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Intraspecific variation of nuclear DNA in *Capsicum annuum* L.

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Abstract. *In situ* nuclear DNA amount varied significantly between 23 varieties of *Capsicum annuum*. Mean values of nuclear DNA showed no correlation with chromosome length. Somatic chromosome number is constant 2n = 24 in all the varieties.

Keywords. *Capsicum annuum*; red pepper; *in situ* nuclear DNA.

1. Introduction

The amount of nuclear DNA in a species is generally constant (Bennett and Smith 1976; Mukherjee and Sharma 1984; Ohri and Khoshoo 1986). The differences in DNA content at an intraspecific level have also been recorded (Raina and Rees 1983; Greenlee *et al* 1984; Banerjee and Sharma 1985; Bennett 1985; Ohri and Khoshoo 1986). Varieties of *Capsicum annuum* L. are extensively cultivated throughout the plains and hills of India. The varieties differ mainly in size, shape and pungency of fruits, in addition to other vegetative characters. The present investigation was carried out to determine intraspecific variation of nuclear DNA, if any, and the extent to which it is correlated with chromosome length.

2. Materials and methods

Seeds of 23 varieties of *C. annuum* were obtained from the National Bureau of Plant Genetic Resources, New Delhi and Globe Nursery and Sutton Seeds, Calcutta.

For studies of chromosome number and size, the seeds were germinated on moist filter paper in petri-dishes. Root-tips of the same age were cut into 2 mm segments and pretreated in a saturated mixture of aqueous para-dichlorobenzene and aesculin solution for 3 h at 15°C, followed by overnight fixation in 1:3 glacial acetic acid ethanol mixture. The root-tips were then hydrolysed in N·HCl for 14 min at 60°C. After thorough washing, the root-tips were stained for 1 h in Feulgen solution prepared from BDH-GURR basic fuchsin, No. 42510 and finally squashed in a drop of 45% acetic acid. Ten well spread metaphase plates were scanned.

For cytophotometric estimation of *in situ* nuclear DNA, healthy, young root-tips were fixed overnight in 1:3 glacial acetic acid ethanol mixture and hydrolysed in N·HCl for 14 min at 60°C. After thorough washing, the root-tips were stained for 1 h in Feulgen solution prepared from BDH-GURR basic fuchsin, No. 42510 and finally squashed in a drop of 45% acetic acid. *In situ* DNA per cell was estimated with the help of a Leitz Wetzlar Aristophot microspectrophotometer following the single wavelength, 550 nm, method of Sharma and Sharma (1980). The 4C nuclear DNA value was calculated from 25 metaphase plates, on the basis of optical density in terms of relative arbitrary units of absorbance, which were then converted to

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absolute unit picogram (pg), by using the 4C nuclear DNA value of 67·1 pg for *Allium cepa* as a standard (Van't Hof 1965).

For statistical analysis of variance test, ANOVA, Sokal and Rohlf's (1973) method and Duncan's new multiple range test were adopted (Harter 1960). The standard correlation coefficient test was carried out between DNA value and chromosome length.

3. Results

3.1 *Chromosome characteristics*

The somatic chromosome number $2n = 24$ was constant in all the 23 varieties of *C. annuum*.

The total chromosome length determined from the mean of 5 well spread metaphase plates in 22 varieties of *C. annuum* showed a mean variation from 90·12–135·44 μm (table 1).

Analysis of variance test (table 4) revealed significant difference. Following Duncan's new multiple range test, varieties BDJ/NKG-88, BDJ/NKG 320, BDJ-1-361 and 613 differ significantly from the rest. Non-significant variation in total chromosome length occur in 6 overlapping groups (table 5).

Table 1.   *In situ* DNA mean and chromosome length in root meristem cells of *C. annuum* varieties.

Varieties	Mean value of 4C nuclear DNA per cell (pg)	Total chromosome length (μm)	Remarks
69/33	18·38	112·01	
106/81–17	18·90	102·45	
G-4	18·90	102·50	
BDJ-11-111	18·98	114·36	Correlation coefficient is non-
K-2553	19·30	111·43	significant between amount of nuclear DNA and total chromosome length.
110/81-16	19·31	105·51	
BDJ/NKG-320	19·40	121·08	
P-272	19·95	103·18	
P-173	20·10	111·71	
BDJ-55	20·41	104·40	$(r = -0.0004; P > 0.05)$
NP-46A	20·50	107·43	
6C-173358	20·58	95·79	
BDJ-613	20·59	135·44	
BDJ/NKG-88	20·65	90·12	
IC-74244	20·71	100·10	
BDJ-1-361	21·00	134·56	
168/26	21·17	107·22	
U8-45	21·34	107·10	
BDJ-388	21·41	100·03	
BDJ-223	21·53	111·55	
U8-137	21·97	102·18	
BDJ/NKG-297	22·98	113·22	

3.2 Amount of nuclear DNA

The 4C nuclear DNA per cell, as worked out from the mean of 25 cells observed, varied from 18·38–22·98 pg between the 23 varieties. Analysis of variance test (table 2) indicated significant difference. Duncan's new multiple range test revealed significant variation in mean DNA value between all the varieties of *C. annuum* with the variety BDJ/NKG-297 with a mean value of 22·98 pg. Four groups with mean DNA value 18, 19, 20 and 21 pg exist among the 22 varieties. Non-significant variations in the value of nuclear DNA exist between 10 groups of varieties (table 3).

Table 2. ANOVA table: Analysis of variation of 4C DNA mean amounts per cell in 23 varieties of *C. annuum*.

Source of variation	Degree of freedom	Sum of squares (SS)	Mean (SS)	F
Between the groups	22	531·1374	24·1426	5·73 ($P<0.05$)
Error or within the groups	552	2324·72	4·2114	

Table 3. Multiple comparison for nuclear DNA amount per cell in varieties of *C. annuum*.

Ordering the sample means	Varieties	Mean value of 4C nuclear DNA per cell	Remarks
1	69/33	18·38	
2	Surjamani	18·59	
3	106/81-17	18·90	
4	G-4	18·90	
5	BDJ-11-111	18·98	Varieties of the order:
6	K-2553	19·30	1–7, 2–8, 5–9, 6–10, 9–16, 10–17,
7	110/81-16	19·31	11–19, 12–20, 13–21 and 18–22
8	BDJ/NKG-320	19·40	are non-significant.
9	P-272	19·95	($P>0.05$)
10	P-173	20·10	
11	BDJ-55	20·41	
12	NP-46A	20·50	
13	6C-173358	20·58	
14	BDJ-613	20·59	
15	BDJ-NKG-88	20·65	
16	IC-74244	20·71	
17	BDJ-1-361	21·00	
18	168/26	21·17	
19	U8-45	21·34	
20	BDJ-388	21·41	
21	BDJ-223	21·53	
22	U8-137	21·97	
23	BDJ/NKG-297	22·98	

Table 4. ANOVA table: Analysis of variation of total chromosome length per cell in 22 varieties of *C. annuum*.

Source of variation	Degree of freedom	Sum of squares (SS)	Mean (SS)	F
Between the groups	21	12357·686	588·46	9·33
Error or within the groups	88	5550·539	63·07	($P<0.05$)

Table 5. Multiple comparison for total chromosome length per cell in varieties of *C. annuum.*

Ordering the sample means	Varieties	Total chromosome length (μm)	Remarks
1	BDJ/NKG-88	90·12	
2	6C-173358	95·79	
3	BDJ-388	100·03	
4	IC-74244	100·10	
5	U8-137	102·18	Varieties of the order: 2–4, 3–9,
6	106/81-17	102·45	5–12, 6–13, 14–19 and 21–22 are
7	G-4	102·50	non-significant
8	P-272	103·18	($P > 0.05$)
9	BDJ-55	104·40	
10	110/81-16	105·51	
11	U8-45	107·10	
12	168/26	107·22	
13	NP-46A	107·43	
14	K-2553	111·43	
15	BDJ-223	111·55	
16	P-173	111·71	
17	69/33	112·01	
18	P-297	113·22	
19	BDJ-11-111	114·36	
20	BDJ/NKG-320	121·08	
21	BDJ-1-361	134·56	
22	BDJ-613	135·44	

Significant correlation could not be obtained between mean amount of nuclear DNA and total chromosome length in the 22 varieties of *C. annuum* where the correlation coefficient (r) was very low, being equal to -0.0004 at 5% level.

4. Discussion

4.1 *Nuclear DNA amount and intraspecific variation*

The results indicate a significant variation in nuclear DNA content in *C. annuum* at intraspecific level, ranging from 18·38–22·98 pg in the varieties 69/33 and BDJ/NKG-297 respectively (table 3). An intraspecific variation of 16–20·68 pg was noted by Owens (1975); but this difference was between the wild and cultivated forms of *C. annuum*, whereas only the 23 cultivated forms of *C. annuum* were investigated. Such intervarietal differences in DNA content, specially those having a wide margin may indicate a genetic distance due to continued accumulation of mutations. The increase in nucleotypic sequences undoubtedly cannot be ruled out (Hutchinson *et al* 1980; Bennett 1982). Such intraspecific variation in nuclear DNA amount is present in varieties of *Lathyrus sativus* but not so marked in other cultivated legumes (Ohri and Khoshoo 1986; Sharma *et al* 1986). The variation of nuclear DNA amount within a single species is an index of genetic variation and it mav play a significant role in genotype diversification (Price *et al* 1983; Banerjee and Sharma 1985). The analysis of repeat DNA content in *C. annuum* may indicate

the extent to which difference in amount is due to repeated sequences. Such repeat sequence analysis would also reveal the sequence affinity, if any, between varieties in a group with the same DNA amount (Flavell 1982; Nagl 1985).

4.2 *Nuclear DNA amount in relation to chromosome length*

Correlation of nuclear DNA amount with chromosome length is non-significant when all the varieties of *C. annuum* is considered together. The varieties K-2553, P-173 and BDJ/NKG-223 have 19·30, 20·10 and 21·53 pg of nuclear DNA respectively with corresponding total chromosome length of 111·43, 111·71 and 111·55 μm respectively (table 1). Similarly varieties BDJ-613 and BDJ/NKG-88 show contrasting total chromosome length of 135·44 and 90·12 μm with nearly same DNA value of 20·59 and 20·65 pg respectively. The range in nuclear DNA, though significant, involves a total amount of 4·6 pg. On the other hand, the range in total chromosome length is 90·12–135·44 μm, that is a difference of 45·32 μm which is also very significant. At an interstrain level a difference of 4·6 pg of DNA is associated with a wide difference of 45·32 μm in chromosome length. It is clear that there is no correlation between the amount of nuclear DNA and chromosome length in the same species. Differences in chromosome length despite nearly similar amount of DNA can be accounted by differential spiralization and consequent condensation of chromosome, a factor which is under genetic control (Mukherjee and Sharma 1986).

5. Conclusion

The present investigation has revealed that the DNA value significantly varies in *C. annuum* at intraspecific level. This variation indicates a possible genetic distance between two groups of varieties. Statistical analysis however, does not reveal any correlation between the amount of DNA and chromosome length in the different varieties. A few of the varieties exhibit marked difference in chromosome length with similar DNA value. Such differences have been attributed to differential spiralization of chromosomes.

Acknowledgement

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Inheritance of polypetalous corolla mutation in sesame

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Abstract. A true breeding mutant having free corolla lobes, designated as polypetalous mutant was obtained in sesame (*Sesamum indicum* Linn.), after irradiating seeds of cv N62–32 with 1·6 kr fast neutrons. The inheritance pattern indicated that the polypetalous corolla is monogenic recessive to the gamopetalous corolla characteristic of sesame and the family Pedaliaceae. After crossing to another induced mutant having gamopetalous corolla with a band of pink coloured dots, a genetic stock with two recessive markers was isolated.

Keywords. Sesame; *Sesamum indicum*; mutation; flower; genetics.

1. Introduction

Sesame (*Sesamum indicum* Linn.) is an edible oilseed crop grown in India and other tropical and sub-tropical countries. In our mutation experiments (Murty *et al* 1985), a mutant (N–29) with free corolla lobes, designated as polypetalous corolla mutant, was isolated in the M_2 generation. It was obtained following 1·6 kr fast neutron treatment to the seeds of cv N62–32 at APSARA reactor of this Research Centre. Gamopetalous, tubular, obliquely campanulate corolla with 4 epipetalous, didynamous stamens is the characteristic of sesame flower (Joshi 1961). In this communication, morphological characters and inheritance pattern of the polypetalous corolla mutation are reported.

2. Materials and methods

The true breeding polypetalous corolla mutant, N–29 was crossed with its parent N62–32 and with Phule Til-1 (PT–1), both having gamopetalous, tubular corolla. It was also crossed to another induced mutant dotted flower (*dtf*) isolated in PT–1, having a band of pink dots inside the tubular corolla extending up to labellum (Murty 1988). Observed segregations in the F_2 and F_3 generations were recorded.

3. Results and discussion

3.1 *Polypetalous mutant*

The mutant N–29 is similar to the parent cultivar N62–32 in the vegetative characters, and can be identified only at the time of flowering. The chief distinguishing character of the mutant is the absence of tubular corolla; the petals remain free as in the case of polypetalous species and are united only at the base (figure 1B), giving a false appearance of polypetaly. In the absence of tubular corolla, the epipetalous stamens in the mutant remain away from the stigma. This

Table 1. Segregation of polypetalous corolla mutant, N-29.

Cross	No. of progenies studied	Without dots — Tubular	Without dots — Polypetalous	With dots — Tubular	With dots — Polypetalous	Ratio	Chi-square	DF	P
F₂ generation									
N62-32 × N-29	11	1417	447	—	—	3:1	1·033	1	0·30-0·50
PT-1 × N-29	38	4070	1297	—	—	3:1	1·990	1	0·10-0·20
N-29 × PT-1	8	896	274	—	—	3:1	1·560	1	0·20-0·30
F₃ generation									
N62-32 × N-29	17	1063	—	—	—	—	—	—	—
	32	1412	432			3:1	2·432	1	0·10-0·20
				X^2 for	17:32	1:2	0·041	1	0·80-0·90
PT-1 × N-29	14	729	—	—	—	—	—	—	—
	29	755	238			3:1	0·564	1	0·30-0·50
				X^2 for	14:29	1:2	0·012	1	0·80-0·90
N-29 × PT-1	17	595	—	—	—	—	—	—	—
	23	647	189			3:1	2·552	1	0·10-0·20
				X^2 for	17:23	1:2	1·512	1	0·20-0·30
Pooled (i) phenotypic and (ii) genotypic segregations									
(i) F₂ and F₃	141	9197	2877	—	—	3:1	1·287*	5	0·90-0·95
(ii) F₃				X^2 for	48:84	1:2	1·019	1	0·30-0·50
F₂ generation									
N-29 × *dtf*	9	399	118	117	36	9:3:3:1	3·178	3	0·30-0·50
dtf × N-29	2	57	18	16	2	9:3:3:1	3·374	3	0·30-0·50
Pooled	11	456	136	113	38	9:3:3:1	2·438	3	0·50-0·70

*Heterogenity chi-square.

results in poor capsule set upon baging the inflorescence but facilitates cross pollination by insects in the open flowers, compared to the other wild type plants with tubular corolla.

3.2 *Inheritance of the mutant trait*

The F_1 hybrids of N–29 × N62–32 and N–29 × PT–1, had tubular corolla,
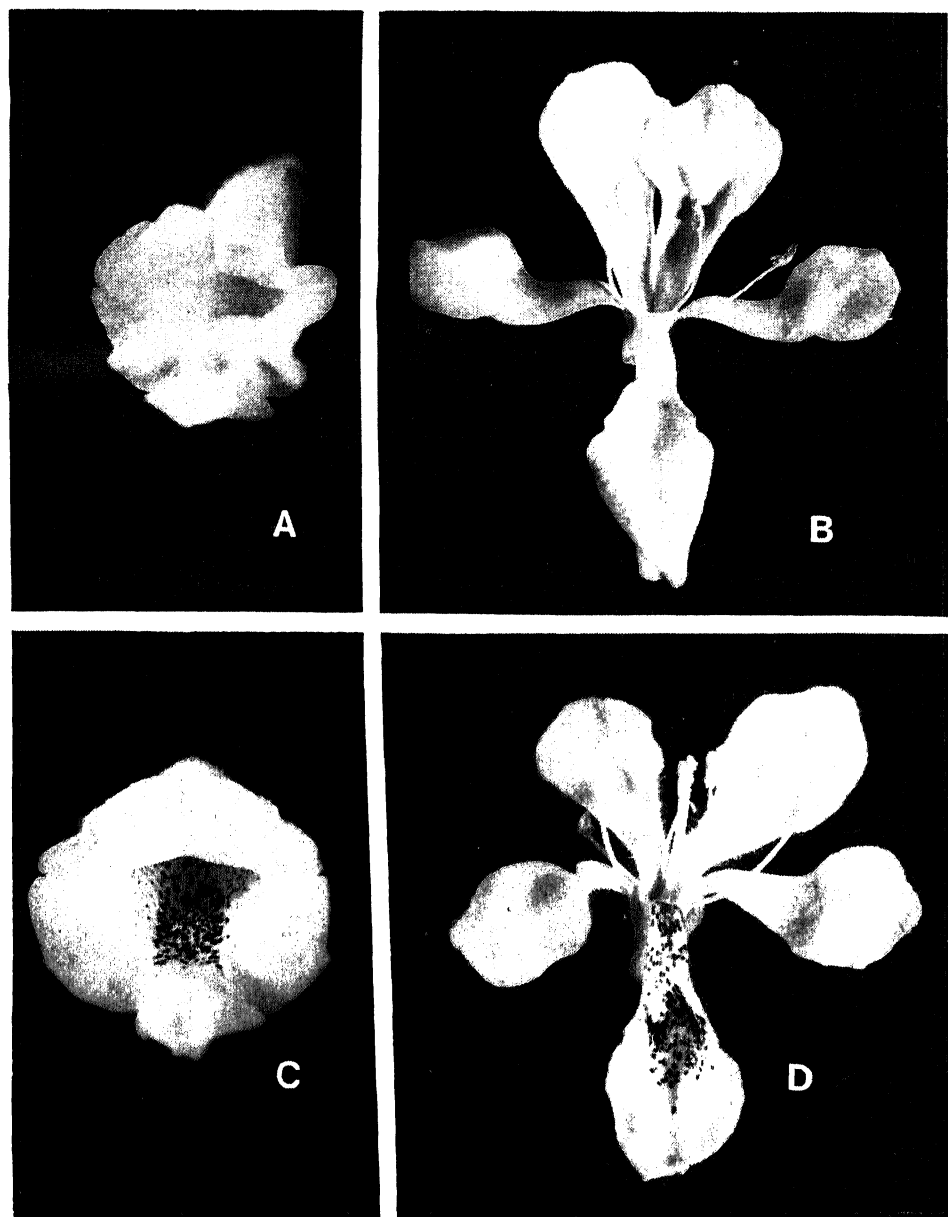
Figure 1. Corolla. **A.** Parent. **B.** Polypetalous mutant. **C.** Dotted mutant. **D.** Recombinant of polypetalous and dotted mutants.

indicating that the mutant character is recessive. The observed segregations in the F_2 and F_3 generations (table 1), for tubular and polypetalous corolla (*pc*) showed that polypetalous character of N–29 is monogenic recessive to the gamopetalous condition.

The corolla character in the F_1 plants of N–29 × *dtf* cross showed wild type, tubular corolla without pink dots. In the F_2 generation, the two corolla characters segregated (table 1) in the ratio of 9 tubular without dots: 3 polypetalous without dots: 3 tubular with dots: 1 recombinant (*pc dtf*) having polypetalous corolla with dots (figure 1).

3.3   *Allelic relationship*

Langham (1947) had reported, star flower, a sterile spontaneous mutant with free petals which did not produce capsules in the absence of hand pollinations. Based on the F_2 observations, he concluded that star flower character was controlled by duplicate recessive genes. The photograph of star flower published by Langham (1947) and the polypetalous mutant, N–29 have close resemblance. However, the present inheritance data, suggests that N–29 is genetically different from star flower. The allelic relationship between the two could not be ascertained due to non-availability of star flower mutant.

The mutant *pc* and the double recessive *pc dtf* would be useful stocks for genetic studies and *pc* could have possible use in hybrid seed production. Extensive hybrid vigour has been reported in sesame by several workers and has been recently reviewed (Osman 1985).

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Effect of carbaryl and 1-naphthol on seedling growth of barley, and on growth and nodulation of groundnut in two soils

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Abstract. Carbaryl at 2·5 ppm in clay and sandy loam soils had no effect on the growth of barley. However, higher concentrations of carbaryl (25 and 100 ppm) were inhibitory and the inhibitory effects were more pronounced in sandy loam soil than in clay soil. Repeated sowing of barley in carbaryl treated soils showed that phytotoxic effects lasted only for 7 days after addition of carbaryl to soils. While carbaryl at 2·5 and 25 ppm had no effect on growth and nodulation of groundnut, at 100 ppm it was inhibitory. 1-Naphthol, the degradation product of carbaryl significantly stimulated seedling growth of barley and nodulation of groundnut.

Keywords. Carbaryl; 1-naphthol; soils; plant growth; nodulation; barley; groundnut.

1. Introuction

Pesticides applied directly or indirectly ultimately reaches the soil. Phytotoxic effects of pesticide residues in soil owing to continuous use of pesticides over a period of time in the same area have been reviewed (Martin 1972). Carbaryl (1-naphthyl, N-methylcarbamate) introduced as a replacement for persistent organochlorine compounds is used as a broad-spectrum insecticide both contact and systemic, against over 150 pests at rates ranging from 0·57–4·5 kg active ingredient/ha. Sometimes, 8–10 applications of carbaryl were given to crops in one season (Singh *et al* 1979). 1-Naphthol is the major degradation product of carbaryl in soil, and is also a metabolic product of naphthalene. We report here the effects of carbaryl and 1-naphthol applied to two soils on seedling growth of barley, plant growth and nodulation of groundnut.

2. Materials and methods

A clay soil (pH 7·3; organic carbon 1·5%; sand 12%; silt 15%; clay 73%) and a sandy lom soil (pH 6·8; organic carbon 0·4%; sand 46%; silt 22%; clay 22%) were collected from Experimental Field Station, Trombay and air-dried before passing through 2-mm sieve. Carbaryl (50 WP) or 1-naphthol was mixed with 10 kg soil aliquots thoroughly to have 2·5, 25 and 100 ppm concentrations.

For studies on seedling height of barley, treated soils were distributed to clay pots (35 cm dia × 15 cm height). Seeds of Hullless barley var. NP 292 were sown in 3 pots for each concentration of chemical and each pot had 30 seeds. At the end of 7 days growth under outdoor field conditions, the seedlings were removed to record the seedling height. Plants grown in soil without chemicals served as control. In order to assess the persistence of phytotoxic effects of carbaryl, second and third

sowings of barley were carried out in 25 and 100 ppm carbaryl treated soils after harvesting the seedlings of the first sowing.

For studies on the growth and nodulation of groundnut plants, the treated soils were distributed in clay pots (10 cm dia × 15 cm height). Fifteen pots were used for each concentration of chemical. Each pot was sown with one healthy seed of groundnut var. Spanish Improved. After 35 days growth, the plants were uprooted gently and washed thoroughly to remove adhering soil particles. Nodules were detached from roots and the numbers were recorded. The shoot portions of the plants were oven-dried at 105°C overnight to record the dry weight. The soils without chemicals served as controls.

3.   Results and discussion

Seedling height of barley was not affected by carbaryl at 2·5 ppm in both clay and sandy loam soils (table 1). Higher concentrations of carbaryl were inhibitory to seedling height. The inhibitory effect was proportional to concentration. Seedling height of barley was inhibited by carbaryl at 25 and 100 ppm more in sandy loam than in clay soil. Carbaryl at 100 ppm reduced the seedling height by 26·6% over control in clay soil while the reduction was 42·5% over control in sandy loam soil.

During second and third sowings (i.e. 14 and 21 days after addition of carbaryl) the seedling growth of barley was not affected in 25 and 100 ppm carbaryl treated soils (table 2). The reduction in seedling height of barley by carbaryl was seen only during the first sowing (7 days after addition of carbaryl). The absence of any phytotoxicity during second and third sowings may stem from decreased persistence of carbaryl in soils. In general, carbaryl had a short half-life (8 days) in soils as determined by chemical methods (Rajagopal *et al* 1984). Our observations also showed that the half-lives of carbaryl in clay and sandy loam soils were 3·5 and 7·8 days respectively (N B K Murthy and K Raghu, unpublished results).

Table 1.   Effect of carbaryl and 1-naphthol on the seedling height of barley in clay and sandy loam soils.

| | Seedling height (cm) | |
| | Soil | |
Soil treatment (ppm)	Clay	Sandy loam
Carbaryl		
Control	6·38	6·28
2·5	6·28	6·59
25	5·78*	4·91*
100	4·68*	3·61*
CD at 5%	0·24	0·23
1-Naphthol		
Control	8·82	8·72
2·5	9·15	9·82*
25	9·31*	10·01*
100	9·97*	9·75*
CD at 5%	0·39	0·33

*Significantly different from control ($P = 0.05$).

Carbaryl at 2·5 and 25 ppm in clay soil stimulated the nodule number of groundnut plants and there was no change in dry weight of seedlings (table 3). However, carbaryl at 100 ppm reduced dry weight of seedlings by 25·9% over control. In sandy loam soil, carbaryl at 2·5 and 25 ppm had no influence on nodule number and dry weight of groundnut plants. However, at 100 ppm of carbaryl nodulation and dry weight of plants were reduced by 48 and 32·6% respectively over controls. The inhibitory effect of carbaryl on plant growth and nodulation have been reported (Rao *et al* 1984; Aggarwal *et al* 1986).

1-Naphthol was stimulatory to seedling height of barley in both clay and sandy

Table 2. Effect of repeated sowings of barley on seedling height in carbaryl treated clay and sandy loam soils.

	Seedling height (cm)	
	Sowing	
Soil treatment (ppm)	2	3
Clay soil		
Control	5·11	6·99
25	5·04	6·80
100	5·07	6·97
CD at 5%	0·39	0·38
Sandy loam soil		
Control	5·36	7·15
25	5·52	7·38
100	5·19	7·47
CD at 5%	0·35	0·41

Table 3. Effect of carbaryl and 1-naphthol on plant growth and nodule number of groundnut plants in clay and sandy loam soils.

	Soil			
Soil treatment (ppm)	Clay		Sandy loam	
	Nodule number	Dry weight seedling (g)	Nodule number	Dry weight seedling (g)
Carbaryl				
Control	36·3	1·27	43·4	1·04
2·5	51·2*	1·16	43·2	1·07
25	45·6*	1·18	43·8	0·94
100	30·5	0·94*	22·3*	0·70*
CD at 5%	6·98	0·15	9·84	0·19
1-Naphthol				
Control	30·8	0·85	49·3	0·89
2·5	39·9*	0·99	62·3*	0·99
25	42·1*	0·99	66·8*	0·96
100	28·3	1·08	51·1	0·98
CD at 5%	6·4	0·16	7·7	0·17

*Significantly different from control ($P = 0.05$).

loam soils (table 1). 1-Naphthol at 2·5 and 25 ppm in both clay and sandy loam soils was stimulatory to nodule number of groundnut plants (table 3). However, it did not change dry weight of groundnut plants. 1-Naphthol at 100 ppm had no influence either on nodule number or dry weight of groundnut plants in both the soils. Gorter (1969) found that 1-naphthol interacted with IAA to stimulate root initiation. Enhanced plant growth by 1-naphthol is known (Dhawan and Nanda 1984; Kakkar and Rai 1986).

The observations presented here showed that the phytotoxic effects by higher concentrations of carbaryl were more pronounced in sandy loam soil than in clay soil. Similar observation on the above soil types was reported when the effect of benzene hexachloride (HCH) on growth and nodulation of groundnut was studied (Murthy and Raghu 1976). It is known that insecticides damaged crops more in light sandy soil than in heavy clay soil (Edwards 1972). Adsorption of carbaryl was more in bentonite clay than in kaolinite clay (Aly and El-Dib 1972) and more pronounced in soils with high organic matter (Carazo *et al* 1979). Carbaryl was known to form significant amounts of bound (unextractable) residues in soils (Murthy and Raghu 1989). The formation of soil bound residues was more with clay soil than sandy loam soil (N B K Murthy and K Raghu, unpublished results). The difference in the phytotoxic effects of carbaryl at higher concentrations between clay and sandy loam soils may be due to the variations in adsorptive capacity and formation of soil bound residues of the two soils studied.

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Cytology of woody members of Rosaceae

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Abstract. Meiotic counts have been made on 16 woody species of the family Rosaceae from Indian forests. Of these, *Photinia notoniana* (n = 17), *Rubus gardnerianus* (n = 28) and *Sorbus foliolosa* (2n = 68) are counted for the first time. Besides, the Indian populations of *Chaenomeles japonica* (n = 17), *Cotoneaster acuminata* and *Eriobotrya japonica* (n = 17) are also worked out for the first time. Intraspecific polyploid cytotypes are recorded in *Pyrus communis* (2x, 3x) and *Rosa leschenaultiana* (2x, 4x). On the basis of chromosomal associations, the tetraploid cytotypes of *Sorbus foliolosa* appears to be alloploid in nature whereas the triploid of *Pyrus communis* show autoploid behaviour. Variation in chromosome number and some pollen sterility in pollen mother cells of meiotically normal diploid individuals of *Cotoneaster acuminata* (n = 17), *Prunus domestica* ssp. *insititia* (n = 8) and *Rosa leschenaultiana* (n = 7) seems to be due to chromatin transfer during cytomixis.

Keywords. Alloploid; autoploid; cytology; cytomixis; Rosaceae.

1. Introduction

Rosaceae, a large family with 100 genera and 2,000 species (Airy Shaw 1973), is cosmopolitan in distribution. However, its members are relatively more abundant in the north temperate regions of India. Most of the 200 Indian species (Hooker 1879) are distributed in various forests. The family is best known for its ornamental value and edible fruits. Besides, some species provide timber of commercial importance and local use. Due to its immense economic importance and large size, the chromosome counts of several species are known today (see Fedorov 1969). However, the chromosome survey has mainly been from the temperate regions and large number of species from the tropical regions remains still uncovered. This is particularly true of the woody species from Indian forests. Some work on the cytology of Indian woody species has been carried out by Malik (1965) and Mehra *et al* (1973) but attention has mainly been on the taxa of western and eastern Himalayan forests. The present communication which covers 16 woody species from the forests of central and southern India, and the Garhwal Himalaya is the part of the project on the cytogenetics of Indian trees. Some cultivated taxa growing in these areas have also been included.

2. Materials and methods

For meiotic studies, appropriate sized flower buds were fixed in Carnoy's fluid with acetic acid component saturated with iron acetate. Anthers were squashed in 1% acetocarmine and desirable preparation were made permanent in euparal. Pollen fertility was estimated with glycero-acetocarmine mixture (1 : 1).

3.   Results and discussion

3.1   *Chaenomeles*

Chaenomeles, an exotic genus, is represented by 3–4 species. *C. japonica* with brick-red flowers is counted to have n = 17 which is a new record for India. The same chromosome number is known to occur elsewhere (Moffett 1931a) and other 3 cytologically known species of the genus.

3.2   *Cotoneaster*

Cotoneaster acuminata a large shrub is best represented in the temperate forests of north-west Himalaya. The chromosome number of the species is not known earlier from india and the present findings of 2n = 34 substantiate the earlier report by Sax (1954). Some pollen mother cells (PMCs) in the presently studied population show cytomictic channels involving chromatin transfer. Both hypo- and hyperploid PMCs are resulted. Existance of some pollen sterility (11%) and pollen grains of variable sizes (32–44 × 30–40 μm) in this taxon might be the consequence of such a chromatin migration.

3.3   *Eriobotrya*

Eriobotrya japonica, a native of China is cultivated in various parts of India for fruits. During meiosis, 17 bivalents are regularly constituted at diakinesis and M-I. In spite of normal bivalent formation and cytokinesis high pollen sterility (38%) is existent in this taxon. The present count of n = 17 which is new for India agrees with earlier reports (Morinaga *et al* 1929; Moffett 1931a, b; Sax 1931; Gadella *et al* 1969). As the other 3 cytologically known species of the genus are also diploid with 2n = 34, the intraspecific cytotype of *E. japonica* with 2n = 32 (Evreinoff 1930) would be an aneuploid derivative of 2n = 34.

3.4   *Photinia*

The genus *Photinia* is distributed in south-east Asia and north America. *P. notoniana* a commonly distributed species in the forests of Nilgiri, Palni and Annamalai hills is counted to have n = 16 which is the first chromosome count for the species.

3.5   *Prinsepia*

Prinsepia is a small genus with 3–4 species, distributed from Himalaya to north China and Formosa. *P. utilis*, the only Indian species is distributed in the forests of Himalaya from 1,200–2,700 m is counted to have n = 16 which agree with the earlier reports. However, an aneuploid cytotype with 2n = 28 exists in south India (Subramanian 1979). The proposal of x = 8 as the base number for the genus (Darlington and Wylie 1955; Mehra *et al* 1973) does not sound well because so far

n = 8 has not been recorded in any of the species of the genus. Provisionally, x = 16 is taken as the base number on the basis of which the species is diploid.

3.6 *Prunus*

Prunus, a genus of 466 species, is well known in horticulture. Of the 19 species represented in india, *P. cerasoides* (*P. puddum*) and *P. cornuta* are the only two commercial timber species of the family in India. *P. puddum*, a small to moderate sized tree is distributed in the Himalaya between 700–2,100 m chiefly in the open forests and on barren slopes. The species is also cultivated in several parts of India. The present count of n = 8 from the Himalaya and Kodaikanal hills agrees with earlier reports.

   P. domestica is mainly cultivated for fruits in almost all parts of India. All the presently studied individuals from Punjab plains (Patiala) and south India (Kodaikanal) show the same chromosome number (2n = 16) which is a new diploid cytotype from India. The meiotic course in the taxa from Kodaikanal show normal meiosis with cent per cent pollen fertility. On the other hand almost all the individuals studied from Patiala show the phenomenon of cytomixis in more than 50% of the PMCs observed. As a result of chromatin migration PMCs with increased (2n = 32) and decreased (2n = 2, 4) chromosome numbers are resulted. Existence of pollen grains of variable sizes (22–36 × 18–30 μm) and high pollen sterility (36%) in this taxon seems to be the consequence of cytomixis.

3.7 *Pyrus*

Of the 23 species recorded from india majority of them are known in horticulture. *P. communis* which is largely cultivated for fruits in different parts of India is studied presently from various sources in Kodaikanal. Both the diploid (2n = 34) and triploid (2n = 51) cytotypes are detected. The meiosis in the diploid taxa is perfectly normal with regular 17 bivalent formation and cent per cent pollen fertility. On the other hand the meiotic course in the triploid is highly irregular and is characterized by the presence of trivalents and univalents, unequal distribution of chromosomes and laggards. The most common type of chromosome distribution during A-I is 25:26. However, PMCs with 24:27 and 22:29 distribution are also existant. Laggards, the number of which varies from 1–7, occur in about 76·2% PMCs at A-I/T-I and 79·4% PMCs at A-II/T-II. In some of the PMCs at A-I, the chromosomes remain scattered and fail to reach at the poles. Consequential to these meiotic irregularities pollen sterility is very high. The taxon appears to be autotriploid in nature because in majority of the PMCs there is a cent per cent trivalent formation.

3.8 *Rosa*

Rosa with 100 species is distributed in the temperate regions of the northern hemisphere with a few species within the tropics. *R. leschenaultiana* a thorny shrub is very widely distributed in the forests of Nilgiri and Palni hills. Cytological samplings of the species from the different forests of Kodaikanal reveal the existence

of intraspecific diploid (2n = 14) and tetraploid (2n = 28) cytotypes. Both the cytotypes are common in these forests and are morphologically indistinguishable. Meiosis in majority of the diploid individuals is normal with regular 7 bivalent formation and cent per cent pollen fertility. However, some PMCs in one of the population show the phenomenon of cytomixis. As a result of chromatin transfer both hypo- and hyperploid PMCs are resulted. Majority of the PMCs in the tetraploid cytotype also show normal meiosis with regular 14 bivalent formation and normal segregation during anaphase. In some PMCs however, chromatin bridges are detected during A-I. Pollen fertility however, is quite high.

3.9   *Rubus*

The genus *Rubus* with 225 species is cosmopolitan in distribution. Of these, as many as 41 species are reported from Indian forests (Hooker 1879). *R. gardnerianus*, a large straggling shrub is very widely distributed in Indian forests.The presently studied populations from the forests of Kodaikanal are found to have n = 28 which is the first chromosome report for the species. In spite of the high chromosome number (2n = 56) and ploidy level (8x), meiotic course in the taxon is perfectly normal with regular 28 bivalent formation and their segregation during A-I. Pollen fertility is also cent per cent.

   R. ellipticus a large shrub is also widely represented in Indian forests. The present diploid count of n = 7 for the species agrees with earlier reports.

3.10   *Sorbus*

The genus *Sorbus* with 100 species is represented in India by 7 species. *S. foliolosa* a shrub or small tree is very widely distributed in the Himalaya from Kashmir to Sikkim between 1,800–3,600 m. The presently studied population from the Garhwal Himalaya reveal the chromosome number to be 2n = 68. The meiotic course in the taxon is highly irregular due to the presence of multivalents and univalents, and laggards. Of the 23 PMCs analysed the most common configuration is of $7_{IV} + 20_{II}$. Laggards are present in 58·4% of the PMCs at A-I/T-I. Incidence of laggards is slightly higher at A-II/T-II. The species which has not been counted earlier is tetraploid on x = 17. On the basis of analysis of chromosome associations, Gill *et al* (1982) suggested the species to be segmental alloploid in nature.

3.11   *Spiraea*

Spiraea a genus of ornamental value is represented by 100 species. All the 3 presently studied species, *S. bella, S. cantoniensis* and *S. lindleyana* are with the same diploid chromosome number (2n = 18) which is in conformity with the earlier records. Intraspecific polyploid cytotypes exist in *S. lindleyana* (2n = 4x = 36, Malik 1965) and *S. cantoniensis* (2n = 3x = 27, Sax 1936; 2n = 4x = 36, Baldwin 1951, Subramanian 1979).

3.12   *Stranvaesia*

Stranvaesia glaucescens, the only Indian species of the genus is found to have n = 17 which agrees with the earlier findings. A few PMCs in the presently studied taxon

show two nucleoli with 2–4 bivalents attached to each of them. Further course of meiosis is regular resulting into 100% pollen fertility.

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Cation budget under terrace agroecosystem in Meghalaya in north-east India

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Abstract. Cation budgeting was done under 4- and 12-year old terraces at higher elevation of Meghalaya (960 m) in north-east India. Cation addition occurred after burning the biomass arising from the weed and the crop residue prior to cropping. While nutrient removal through weeds was more under 12-year old terrace than under 4-year old one, the reverse was true for that removed by crop. Nutrient deficit, particularly potassium, was obvious under 12-year old terrace. Decline in soil fertility and increase in weed potential are implicated in the reduced crop yield.

Keywords. Terrace; cation budget; biomass; soil fertility.

1. Introduction

Slash and burn agriculture (jhum) is the chief land use of the north-eastern hill region (Ramakrishnan *et al* 1981; Ramakrishnan 1985) and this agricultural activity is the chief one in other areas of the humid tropics elsewhere (Nye and Greenland 1960; Ruthenburg 1976). In recent times, the slash and burn agriculture cycle (the length of the fallow period before the land is again cleared for cropping) has become extremely short (4 to 5 years) due to increased population pressure and reduced land area (Mishra and Ramakrishnan 1981; Toky and Ramakrishnan 1981a). One of the suggested alternatives has been settled farming on terraces, though this has not found acceptance by the people. However, in some selected areas where the soil is sufficiently deep and well formed, terracing has been continually practised over some time, largely by the immigrant Nepalis as at Nayabunglow, in Meghalaya in north-east India. Among the many factors that contribute to the large-scale rejection of this alternative land use system (Patnaik 1988), nutrient supply to the agroecosystem is an important one. Under terrace system the slash of the weed biomass and crop residue arising from the previous cropping is slashed and burnt which results in release of cations in one single flush. However, under continuous cropping on the terraces cations are depleted through heavy leaching. Of all the cations, the loss of potassium, is most pronounced as shown in our studies in north-east India (Toky and Ramakrishnan 1981b; Mishra and Ramakrishnan 1983; Swamy and Ramakrishnan 1988) and also shown by others (Nye and Greenland 1960). In terraced plots, even though surface run-off would minimized, nutrient leaching would still be a major carrier under the high rainfall condition in north-east India. Therefore the present paper considers the budgeting of cations under terraces maintained for 4 and 12 years under continuous cropping, by the Nepalis. The objective is to calculate the role of cations in the sustenance of this land use system by the immigrant Nepalis alone in this region.

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2. Study area

The study area at Nayabunglow is located about 30 km north of Shillong (25° 45″ N and 91° 54″ E) at an altitude of about 960 m, in the Khasi hills of Meghalaya. The precambrian rocks are represent by gniess, schists and granites. The soil is a red sandy loam of laterite origin. The pH ranged from 5 to 6. The angle of slopes ranged from 20°–40°.

The climate of the area can be divided into 4 more or less marked seasons, (i) the monsoon season of heavy rainfall during May–September, (ii) a transitional period of low rainfall during October–November, (iii) a winter season during December–February and (iv) a windy dry summer during March–April. The average rainfall during the study period was 1800 mm. The average maximum and minimum temperatures during the monsoon season were 28·6 and 17·1°C respectively, and during the winter periods these were 21·3 and 4°C respectively.

3. Description of agroecosystem

3.1 *Terrace agroecosystem*

Terrace cultivation resembels slash and burn agriculture (jhum) in that some slash burning is done followed by mixed cropping. The weed biomass produced between December–March when the land is fallowed is slashed and the weed residue and the crop residue from the previous croppings are burnt before crop introduction in April. On younger terrace, two croppings are done in a year, a mixed cropping between April–August followed by monocropping of *Eleusine coracana*. In older terraces, mixed cropping alone is done. Organic manure is applied at the rate of 2550 kg ha^{-1} yr^{-1} before crop sowing in April. *Zea mays*, *Vigna sinensis*, *Phaseolus vulgaris* and *Cucurbita maxima* are sown simultaneously followed by sequential harvesting, as the crop matures, between August and December. Hand hoeing is done to remove weeds that pose a problem. This weed biomass gets recycled into the plots.

4. Methods

Terrace plots of 4- and 12-yr old (each with 3 replicates) were identified at Nayabunglow 30 km north of Shillong at an elevation of 960 m, in Meghalaya in north-east India. While selecting the plots, similar aspect and topographic conditions were ensured. Direct fall through precipitation was collected from 10 random points in each plot. Soil sampling up to a depth of 40 cm was done by using a core sampler at 15 random points on each plot at 3 times during the year: (i) a day before burning the slash prior to cropping, (ii) a day after the burn and (iii) at the end of cropping.

The slash (weed and crop residues) and organic manure are uniformly spread out in each plot. In order to calculate the amount of slash burnt and the organic manure input into the agroecosystem, ten 1 m^2 quadrats were randomly laid in each plot; values represent the mean of these 10 observations in each replicate plot.

The nutrient input into the agroecosystem was then quantified. Nutrients

removed through crop thinning, crop uptake, weed uptake and recycling through weeds ploughed back into the system were all based on 10 random observations in each plot, using 1 m^2 quadrats.

For studies pertaining to cation losses through sediment and run-off water, loss from a confined area of 1 × 10 m was collected in large collectors and sampled periodically for chemical analysis. For the study of percolation loss of cations, zero tension lysimeters (Buckman and Brady 1960). In each of the 3 replicate plots, 15 lysimeters were placed at random to obtain the mean. Soil was cut vertically at each site to expose the profile. A small tunnel was excavated at a depth of 40 cm (the depth to which most roots penetrate) and the lysimeter 30 × 30 × 15 cm was placed inside it. By pressing from below, the rim of the lysimeter was firmly inserted in the undisturbed soil above. The percolated water was tapped out from the lysimeter, from time to time for analysis. The observations were based on 5 replicates in each plot. A few drops of 40% formaldehyde was added to the samples to stop biological activity immediately after collection.

The amount of nutrients present in the soil pool (kg ha^{-1}) was calculated to a depth of 40 cm using soil bulk density estimates calculated for each site, at depths of 0–7, 7–14, 14–28 and 28–40 cm, considered separately. Bulk density or volume weight (the quotient of the over dry weight at 105°C of the soil to the total volume it occupies in each field) was determined from the air dry mass of a known field volume of soil.

The soil was air dried and plant samples were oven dried at 60°C for 48 h, powdered and passed through 0·2 mm sieve and stored in glass jars for subsequent analysis by procedures given by Allen *et al* (1974). Plant samples were wet digested with triple acids (perchloric acid, nitric acid and sulphuric acid) and soils were extracted with 1 M ammonium acetate solution at pH 7. Thus calcium and magnesium were estimated by EDTA titration and potassium by flame emission method.

While calculating the nutrient budget between the pre-burn and the post-burn stages, nutrient addition through weed/crop residues during the intervening fallow period that were burnt were considered. Calculations of the amount of nutrients (potassium, calcium and magnesium) gained due to slash burning are based on the differences of that element present in the soil up to a depth of 40 cm between pre-burn (a day before burn) and that present in the soil a day after the burn. Input and output of elements for each plot were calculated on the basis of the amount of that particular input/output and the concentration of the element in it.

5. Results

Potassium and magnesium in the pre-burn and post-burn soil were markedly higher ($P < 0.005$) under 12-yr old terrace than under 4-yr old terrace (table 1). Addition of cations through weed residue was more under 12-yr old terrace than under 4-yr old one. Addition through crop residue before the burn was not very different in 4- and 12-yr old terraces. The net gain of potassium and magnesium in the post-burn soil pool was more under 4-yr old terrace than under 12-yr old one and the reverse was true for calcium ($P < 0.01$).

During the first cropping, more cation was immobilised by the weeds and more addition occurred through them under 12-yr old terrace than under 4-yr old one

Table 1.   Gain of cations through fire (kg ha^{-1}) under terrace agroecosystem in north-east India.

| | Terrace age (yr) | | | | | |
| | 4 | | | 12 | | |
	Potassium	Calcium	Magnesium	Potassium	Calcium	Magnesium
Pre-burn soil pool	510 ± 25.1	2122 ± 150	792 ± 43.1	982 ± 34.1	1385 ± 57.6	1086 ± 47.8
Addition through						
Weed residue	23 ± 2.4	10 ± 1.1	13 ± 1.07	38 ± 3.1	26 ± 1.6	27 ± 2.5
Crop residue	57 ± 5.6	39 ± 3.02	32 ± 2.9	53 ± 3.1	36 ± 2.5	32 ± 2.5
Post-burn soil pool	797 ± 49.04	2272 ± 141.5	1043 ± 37.83	1155 ± 58.9	1581 ± 32.16	1308 ± 26.52
Net gain	207	101	205	82	134	165

(table 2). However, the proportional contribution through grasses was more ($P < 0.01$) under 4-yr old terrace than under 12-yr. During the second cropping on 4-yr old terrace, the cation recycled through weeds originated from the previous cropping season. Further, during the second cropping, the proportional contribution by grass species was more ($P < 0.01$) than through dicots.

Total nutrient removal by edible and non-edible components of crop species was higher ($P < 0.01$) under 4-yr old terrace than under 12-yr old one (table 3). If the second cropping done under 4-yr old terrace is excluded the reverse was found to be the case. *E. coracana* under 4-yr old terrace removed a larger proportion of potassium during the second cropping season than other species of the first cropping phase. Removal of nutrients through non-edible component for a given species was significantly higher ($P < 0.01$) than through edible parts.

The input/output pattern for cations is given in table 4. While there was a net gain of potassium under 4-yr old terrace, there was loss under 12-yr old one; the reverse was true for calcium. Magnesium gain was more or less similar under 4- and 12-yr old terraces. In general, the input and output totals were more under 4-yr old terrace than under 12-yr.

Nutrient status both before burn and after cropping was higher ($P < 0.01$) under 12-yr old terrace than under 4-yr old one, the exception being calcium (table 5). A net loss in calcium under 4-yr old terrace and a similar loss for potassium under 12-yr old terrace were noted, while others showed a net gain at the end of cropping.

6.   Discussion

Terrace cultivation introduced as an alternative land use to replace jhum is largely practised by non-tribal immigrant Nepalis in this region. Apart from the input of organic fertilisers such as cow dung and compost, for terrace cultivation slash and burn operation associated with shifting agriculture (Nye and Greenland 1960; Spencer 1966; Ruthenburg 1976; Ramakrishnan 1984) is also done. However, the slash is largely the crop and weed residues from the previous cropping season. While massive losses of nitrogen are associated with the burn, a substantial increase in exchangeable cations occurred after the burn. Though the budget up to 40 cm depth of the soil profile was done, the changes that occurred due to the burn was

Table 2. Contribution through weed (kg ha^{-1}) during cropping under terrace agroecosystem in north-east India.

| | Terrace age (yr) | | | | | | | |
| | 4 | | | | 12 | | | |
	Biomass	Potassium	Calcium	Magnesium	Biomass	Potassium	Calcium	Magnesium
First cropping								
Weed biomass	2050±31·8	32±3·1	20±1·8	23±1·9	2759±151·6	44±2·3	37±2·8	43±2·0
	(741±20·7)	(10±0·9)	(6·2±0·3)	(8±0·7)	(255±9·2)	(5±0·4)	(2±0·2)	(3±0·3)
Weed recycled during cropping	548±8·7	8±0·3	6±0·2	7±0·2	1009±12·6	15±0·9	12±0·4	13±0·9
	(167±3·8)	(3±0·1)	(2±0·1)	(2±0·2)	(143±4·7)	(2±0·2)	(1±0·09)	(1·2±0·03)
Second cropping								
Weed slash ploughed in prior to second cropping	1503±29·6	23±3·5	14±1·7	16±1·8	—	—	—	—
	(574±23·2)	(74±0·9)	(5±0·3)	(6±0·7)				
Weed biomass	1038±22·6	17±1·2	11±0·7	12±0·9	—	—	—	—
	(628±19·1)	(7·1±0·4)	(5·2±0·3)	(54±0·2)				

Values in parantheses are for grasses.

Table 3. Cation removal (kg ha^{-1}) through edible and non-edible crop biomass under terrace ecosystems in north-east India.

| | Terrace age (yr) | | | | | |
| | 4 | | | 12 | | |
	Potassium	Calcium	Magnesium	Potassium	Calcium	Magnesium
Grains and pulses						
Zea mays*	7·8 (42·4)	11·5 (34·6)	17·8 (37·1)	11·8 (49·3)	12·43 (27)	15·5 (23·8)
Eleusine coracana	18·9 (49·1)	33·1 (37·1)	16·6 (36·1)	—	—	—
Vigna sinensis	0·02 (0·9)	0·02 (0·34)	0·02 (0·6)	0·02 (2·02)	0·012 (0·5)	0·02 (0·8)
Phaseolus vulgaris	0·18 (0·7)	0·2 (0·26)	0·28 (0·5)	—	—	—
Total	26·9 (93·1)	44·82 (72·3)	34·7 (74·3)	11·8 (51·3)	12·44 (27·5)	15·6 (24·5)
Leafy and fruit vegetables						
Momordica dioica	0·03 (0·4)	0·02 (0·2)	0·03 (0·02)	0·013 (1·3)	0·01 (0·45)	0·02 (0·4)
Cucurbita maxima	1·38 (0·2)	0·03 (1)	0·05 (0·01)	2·5 (0·2)	0·04 (0·04)	0·05 (0·06)
Hibiscus sabdariffa	0·009 (0·03)	0·005 (0·004)	0·01 (0·02)	—	—	—
Total	1·4 (0·6)	0·06 (0·3)	0·09 (0·06)	2·5 (1·4)	0·05 (0·5)	0·1 (0·5)
Tuber and rhizomes						
Ipomoea batatus	—	—	—	— (1·6)	— (0·3)	— (0·4)
Colocasia antiquorum	—	—	—	— (0·2)	— (0·003)	— (0·1)
Total	—	—	—	— (1·8)	— (0·3)	— (0·5)
Grand total	28·3±2·8 (93·7±6·7)	44·9±4·6 (72·6±7·3)	34·8±3·3 (74·4±6·9)	14·3±1·6 (54·5±4·7)	12·5±0·7 (28·3±1·2)	15·6±1·1 (25·5±1·6)

Values in parantheses are for non-edible component.
*Grown as a 2nd crop of the mono cropping system.

Table 4. Input/output patterns for cations (kg ha^{-1}) under terrace agroecosystems in north-east India.

	Terrace age (yr)					
	4			12		
	Potassium	Calcium	Magnesium	Potassium	Calcium	Magnesium
Inputs						
Precipitation	4·1	7·4	7	4·1	7·4	7
Addition through fire	207	101	205	82	134	165
Thinned crop biomass	6·8	4·1	3·8	5·01	2·0	1·8
Weeds ploughed back during						
First crop	23·4	14·4	15·9	14·6	12·2	13
Second crop	8·3	5·9	7·1	—	—	—
Organic manure	16·6	10·2	9·1	14·1	8·7	7·7
Total (a)	266 ± 12·0	143 ± 6	248 ± 8·1	120 ± 6·3	164 ± 8·1	195 ± 15·4
Outputs						
Sediment	2·1	3·6	4·1	5·1	4·6	5·1
Run-off	20	7·1	8·1	19·3	13·3	6·1
Percolation	7·9	2·7	2·5	6·1	3·1	3·4
Weed removal during						
First crop	31·7	20·3	23·0	44·3	36·8	43
Second crop	16·8	10·8	11·9	—	—	—
Crop removal during						
First crop	60·8	51·3	60·3	73·8	42·8	43
Second crop	68·0	70·2	53	—	—	—
Total (b)	207 ± 13·2	166 ± 11·3	163 ± 8·3	149 ± 9·9	101 ± 10·9	101 ± 8·4
Net differences (a–b)	+ 59	− 23	+ 85	− 29	+ 63	+ 94

Table 5. Net change in nutrients (kg ha^{-1} yr^{-1}) under terrace agroecosystem in north-east India.

	Terrace age (yr)					
	4			12		
	Potassium	Calcium	Magnesium	Potassium	Calcium	Magnesium
(a) Soil pool before burning	510 ± 25·1	2122 ± 150	792 ± 43·1	982 ± 34·1	1385 ± 57·6	1086 ± 47·8
(b) Soil pool at the end of the cropping	552 ± 50	1473 ± 50·5	1307 ± 57·7	643 ± 45·0	1670 ± 120·3	1547 ± 30·1
(a–b) Net difference	42	649	574	339	285	461

confined only to the first 0–7 cm layer. All the increase that occurred in the soil pool could not be accounted by the input through ash. Obviously, mobilization of cations into the exchange pool after the burn may be an important factor and may be related to increased cation exchange capacity of the soil and the consequent

interchange between non-exchangeable to exchangeable forms due to burning (Stromgaard 1984).

With a higher weed potential on older terraces, the biomass recycled through this component of the agroecosystem is two times more under a 12-yr old terrace than under 4-yr old one, during the first cropping. During the second cropping under 4-yr old terrace, the weed slash from the previous cropping phase is just ploughed in and not subjected to burn. If this is considered together with the weed biomass put back during the first cropping phase, the weed recycled becomes more under 4-yr old terrace than under 12-yr old one. Because of higher weed potential of the site under 12-yr old terrace, the nutrient removal by the weed population was generally more compared to 4-yr old one, inspite of two croppings under the latter situation. In contrast to this, crop removal of cations was markedly higher under 4-yr old terrace than in the older one.

The above discussed differences between the two terrace systems when considered along with nutrient losses related to hydrology (where losses were more under older terraces because of poor physical quality of the soil) the input was higher than the output for a labile element such as potassium under a 4-yr old terrace. This may be related to drastic decline in the nutrient status of the soil under continuous cropping (Asamoa 1980; Cowgill 1961; Sanchez 1976) which results in decline in crop production. The negative value for calcium under 4-yr old terrace may be related to high uptake by *E. coracana* during second cropping.

The results presented here suggests that continuous cropping on terraces apart from adversely affecting soil fertility also results in increased weed potential of the site, both of which contribute to reduced crop yield. However, at Nayabunglow, the soil is deep and well developed and therefore terraces can be sustained over a long time period but with sustained input of organic manure particularly cow dung which the Nepalis alone can afford because they maintain cattle. Tribal farmers are unable to maintain terraces in the absence of organic manure availability. They prefer to do shifting agriculture instead. However, elsewhere in the region where the soil is poorly developed terrace farming is not viable (Ramakrishna 1984) inspite of availability of organic manure.

Acknowledgements

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Pollination ecology of *Moringa oleifera* (Moringaceae)

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Abstract. At Visakhapatnam (17°42′N–82°18′E), *Moringa oleifera* Lam. flowers twice a year, once during February–May and again during September–November. Both geitonogamous and xenogamous pollinations produce fruit, but the latter mode is superior. The flowers are zygomorphic and gullet type. They open during 0300–1900 h, and are visited only by diurnally active insects during 0600–1500 h. Bees are the dominant foragers, of which *Xylocopa* and *Amegilla* carry pollen on the head and/or thorax to effect nototribic pollination. *Xylocopa* was more frequent and proved to be the major pollinator.

Keywords. Pollination; *Moringa*; bees; *Xylocopa*; *Amegilla*.

1. Introduction

Moringa oleifera Lam., popularly known as the drumstic tree, is indigenous to north-west part of India, and thrives best under the tropical insular climate of south India (Anon. 1962). The tree is valued mainly for the tender pods used as vegetable. It is propagated by seed or from cuttings, but it is stated that the progeny resulting from seed is of inferior quality (Anon. 1962). In order to produce genetically superior stocks, it is necessary to have a detailed knowledge of the reproductive biology including breeding behaviour and pollination ecology. Such information is virtually lacking not only for *M. oleifera* but also for most tropical tree species (Bawa 1976). Grant (1950) listed *Moringa* flowers under bird pollination. Salim Ali (1932) recorded sunbirds and bees visiting *M. oleifera*. As part of a larger study on the pollination ecology of tropical trees, we recorded *Xylocopa*, the carpenter bee, actively foraging on *M. oleifera*. We describe here the breeding system, and the relative efficacy of the carpenter bee and of other visitors as pollen vectors of the drumstick tree.

2. Materials and methods

M. oleifera cultivated in the backyards of residential houses at Siripuram (SP), Ram nagar (RN), II town police station (II TPS), Isakathota (IT), BVK College (BVKC)—all the sites located at Visakhapatnam (17°42′N and 82°18′E), were chosen for the study. Dates of first and last flowering and the duration of peak flowering of 46 trees were registered. Daily flower production on 25 randomly selected inflorescences, and the time of daily anthesis and anther dehiscence in relation to the prevailing weather were recorded. Pollen number per anther was determined from 25 flowers distributed over different trees following Subba Reddi and Reddi (1986). Pollen viability and stigma receptivity were studied as per the method described by Subba Reddi and Reddi (1984). Pollen-ovule ratio was

computed after Cruden (1977). The sugars in the nectar were determined by paper chromatography (Harborne 1973). Amino acid and protein presence in nectar was demonstrated as by Baker and Baker (1973). The operation of a particular breeding system was revealed by hand-pollination of 50 flowers and observing them for fruit formation. The extent of fruit set, seed set and fecundity was assessed through observing 1000 flowers for fruit development after their pollination.

Visitors' activity at the flowers, the number of flowers they visited in a minute time, the time (s) a visitor spent at the flower, pollen pick-up by the visitors and the pollen transferred to stigma under forager activity were studied following Subba Reddi *et al* (1983) and Reddi and Subba Reddi (1983). The nomenclature used for the insects is the one given by the Identification Services, Commonwealth Institute of Entomology, London.

3. Results

3.1 *Floral dynamics*

3.1a *Blooming phenology:* While it is likely to find some trees in flower at any time of the year most bloom during February–May and again during September–November, the former season being more intense. Based on flowering intensity, each season could be subdivided into initial, peak and final phases. The limits of these phases observed in the 2 seasons for 46 plants spread over 5 study sites are given in table 1. It is evident that the individual trees vary much in the duration of their flowering. During February–May, the length of flowering varied between 39–60 days, with a mean of 51 days, while during September–November, it ranged from 52–71 days, with a mean of 60 days.

The paniculate cyme over a period of 3–14 ($\bar{x}=7$) days produces 5–50 ($\bar{x}=20$) flowers with day 3 recording a relatively larger number of open flowers. It was found that flower buds prematurely drop off in the 2 seasons, about 30% during February–May and 40% during September–November. Within the season, the bud drop is less during an initial phase, and it increases with the progress of the season.

3.1b *Anthesis and anther dehiscence:* Open flowers are available during 0500–0900 h in association with a temperature range of 27·3–29·3°C and RH 68–78%. Anthers dehisce at anthesis by longitudinal slits. Overcast sky and/or rainy weather may delay the process for 30 min. Flower life lasts for 72 h.

3.1c *Pollen and stigma characters:* Pollen grains 35 μm, spheroidal, surface oily and sticky. Their number per anther averaged 4920 ($R=4720$–5600) and per flower 246000. Sterile grains per anther averaged 300 ($R=220$–400). In 100% sucrose solution, fresh pollen gave 100% germination, 24 h old ones 72% and 72 h old ones 30%; afterwards zero. Hand-pollinations with 24, 48, 62 and 66 h old pollen resulted respectively 72, 60, 32 and 12% fruit set. Grains stored further were ineffective.

The stigma becomes receptive after 24 h of anthesis, continues to be so for 48 h, then turns light brown. Hand-pollinations of freshly receptive stigmas gave 100% fruit set, those of 24 h old ones 72%, and those of 48 h 36%. Pollen-ovule ratio approximated to 1070:1.

Table 1. Flowering phenology of *M. oleifera* in 1983.

Study site	No. of plants	February–May season			Total no. of days	September–November season			Total no. of days
		1st flower	Peak	Last flower		1st flower	Peak	Last flower	
Siripuram	4	20 Feb.–9 Mar.	10 Mar.–5 Apr.	6 Apr.–15 Apr.	55	5 Sep.–19 Sep.	20 Sep.–20 Oct.	21 Oct.–10 Nov.	67
"	2	20 Feb.–4 Mar.	5 Mar.–30 Mar.	31 Mar.–15 Apr.	55	1 Sep.–14 Sep.	15 Sep.–10 Oct.	11 Oct.–5 Nov.	66
"	3	25 Feb.–9 Mar.	10 Mar.–5 Apr.	6 Apr.–25 Apr.	60	15 Sep.–30 Sep.	1 Oct.–30 Oct.	31 Oct.–15 Nov.	62
"	2	5 Apr.–14 Apr.	15 Apr.–15 May	16 May–20 May	46	5 Dec.–19 Dec.	20 Dec.–15 Jan.	16 Jan.–30 Jan.	56
Ram nagar	2	20 Feb.–4 Mar.	5 Mar.–30 Mar.	31 Mar.–20 Apr.	60	15 Sep.–30 Sep.	1 Oct.–25 Oct.	26 Oct.–5 Nov.	52
"	2	1 Mar.–14 Mar.	15 Mar.–5 Apr.	6 Apr.–15 Apr.	46	1 Sep.–14 Sep.	15 Sep.–10 Oct.	11 Oct.–25 Oct.	55
II Town police station	7	5 Feb.–14 Feb.	15 Feb.–10 Mar.	11 Mar.–31 Mar.	55	5 Sep.–19 Sep.	20 Sep.–20 Oct.	21 Oct.–5 Nov.	62
"	3	5 Feb.–14 Feb.	15 Feb.–10 Mar.	11 Mar.–15 Mar.	39	1 Sep.–19 Sep.	20 Sep.–20 Oct.	21 Oct.–10 Nov.	71
Isakathota	5	1 Mar.–14 Mar.	15 Mar.–5 Apr.	6 Apr.–15 Apr.	46	5 Oct.–19 Oct.	20 Oct.–15 Nov.	16 Nov.–30 Nov.	57
"	5	10 Apr.–24 Apr.	25 Apr.–20 May	21 May–31 May	52	5 Dec.–19 Dec.	20 Dec.–15 Jan.	16 Jan.–25 Jan.	52
BVK College	3	5 Feb.–24 Feb.	25 Feb.–15 Mar.	16 Mar.–25 Mar.	49	25 Sep.–9 Oct.	10 Oct.–5 Nov.	6 Nov.–20 Nov.	57
"	2	10 Feb.–14 Feb.	15 Feb.–15 Mar.	16 Mar.–25 Mar.	44	1 Oct.–14 Oct.	15 Oct.–15 Nov.	16 Nov.–30 Nov.	61
"	3	5 Jan.–19 Jan.	20 Jan.–10 Feb.	11 Feb.–5 Mar.	60	20 Aug.–14 Sep.	15 Sep.–10 Oct.	11 Oct.–20 Oct.	62

Flowering period during February–May ($\bar{x}$) = 51 days; R = 39–60 days; SD = 6.57.
Flowering period during September–November ($\bar{x}$) = 60 days; R = 52–71 days; SD = 5.88.

3.1d *Nectar dynamics*:   Nectar secretion begins with anthesis. It amounted to
1·3 μl per flower. Sugar concentration varied with the time of day, it was 18% at
0500 h, 14% at 0800 h, 12% at 1000 h, 9% at 1400 h and 5% at 2000 h. Glucose is
the dominant sugar, with fructose and sucrose in traces. Proteins and amino acids
are present as indicated by the nectar spots on chromatographic paper, showing
violet and blue colour on treatment with ninhydrine and bromo-phenol-blue
respectively.

3.1e *Breeding behaviour*:   Apomixis and autogamy experiments did not yield any
fruit. Hand-pollinations with xenogamous pollen gave 100% fruit set, 81% seed set
and 9% fecundity, while with geitonogamous pollen the respective rates were 62, 64
and 6%.

3.1f *Fruiting pattern*:   Natural fruiting was found to be relatively low. It was 15%
during February–May season, 11% during September–November. Within each
season, it was relatively larger in the initial phase and decreased as the season
advanced.

3.2 *Flower visitor dynamics*

3.2a *Composition and relative abundance*:   Nine hymenoptera, 1 dipteran and 7
lepidoptera were observed to forage at the flowers during the study period (table 2).
All the 17 species did not appear in the 2 flowering seasons, at the 4 sites and in the
2 years of study. The bee species *Apis florea*, *A. cerana indica*, *Trigona* sp., *Xylocopa*
sp. and the ant *Camponotus* sp. made 94% of total visits. The butterfly *Barbo bevani*
was consistent in its visits, while others were occasional.
   Of the bee species, the visits of *Xylocopa* (*X. latipes* and *X. pubescens*) were more
numerous and constituted ca. 57% of total bee visits; those of *Apis florea* made
21%, *A. cerana indica* 12·6%, *Trigona* sp. 8% and *Amegilla* sp. 1·2%. This order
prevailed in the 2 seasons and at the 2 sites. Of the butterfly visits, those of
B. bevani constituted a larger percentage (51%). The diurnal moth appeared on 3 of
8 census days and its visits were sporadic.

3.2b *Diurnal activity pattern of flower visitors*:   The 17 visitor species are day
active. On 3 March 1983 at SP site, bee activity started briskly at 0600 h, it then
ebbed in the next 4 h and then resurged in the next 2 h reaching a moderate peak at
1200 h, from then onwards it began to decline, rather gradually between
1200–1300 h and then rather suddenly up to 1500 h, when it ceased.
   At the II TPS site, on 5 March 1983, bee activity began at 0600 h, showed a
slight increase in the next hour, then ebbed, but again increased at 0900 h, followed
by a sudden fall and continued up to 1400 h. Then on, it was relatively higher over
the next 3 h.
   It was observed that in unbagged flowers nectar occurs in traces. Hence, the
pattern of flower visitor activity might not be totally influenced by nectar secretion
pattern. However, it could be influenced by the coexisting plant species in bloom.
Thus it was observed that during March 1983 the patches of *Antigonon leptopus*
Hook. and Arn. occurring at these sites were in full bloom. The bee species under
consideration did not confine entirely to *M. oleifera*. During the hours of low

activity from 0700–1000 h at SP site and during 1000–1400 h at II TPS, they were observed to concentrate more on *A. leptopus*.

A totally different diurnal activity pattern of bees was observed in September–November season, in that no troughs were evident between morning and afternoon peaks. During this period also *A. leptopus* was present in flower but not in high intensity. As a result, there was no alternate resource for the bees and therefore they concentrated on *M. oleifera*. Thus, the activity profile showed an increase from 0600 h to a high in the next 4 h and then exhinited a gradual decline until 1700 h at the SP site on 10 October 1983. A similar pattern was evident at the II TPS site on 29 September 1983.

3.2c *Relative mobility of insect visitors:* Table 3 gives the data on the number of flowers visited by the insect foragers in a unit time (min) and the time(s) they spent at a flower. Of the 12 species for which such data were collected, *Xylocopa* proved to be more mobile, in that they covered a larger number of flowers per minute than other species.

3.2d *Foragers' behaviour at flowers:* The flowers being zygomorphic are well suited for visitation by *Xylocopa*. When the carpenter bee visited a flower for nectar, it alighted on the reflexed petals, and its weight depressed them a little; the essential organs then brushed against the dorsal side of the head depositing the pollen nototribically. These bees were not seen confining to a particular tree and flew from tree to tree. *Apis* sp., *Musca* sp., *Trigona* sp., after landing on the inflorescence, walked in to the flower and took nectar from lateral sides and did not make any meaningful contact with stamens and stigma. *Macroglossum gyrans* hovered in front of the flower and inserted its proboscis, in so doing the tongue rarely contacted stigma and stamens. The butterflies probed the flower from the side without contacting the essential parts.

3.2e *Pollen pick-up by the various flower visitors:* Pollen grains were present only in the body washings of *Xylocopa latipes*, *X. pubescens* and of *Amegilla* sp. Their numbers were respectively 20–1320 ($\bar{x} = 178$), 45–250 ($\bar{x} = 126$) and 0–4 ($\bar{x} = 1$).

3.2f *Pollen depletion from anthers and depositions on stigma under foragers' activity:* Both depletion and deposition of pollen were contingent upon the insect activity. They were low at 0800 h gradually increased to a peak at 1000 h when the visitors were very active, again they were low until 1800 h. It was found that of the total pollen produced per anther, 63% remain adhered to the pollen sacs.

4. Discussion

M. oleifera at Visakhapatnam exhibits a flowering frequency of two (Ewusie 1980). With most tropical tree species, a flowering frequency of more than one appears to be a common feature (Groat 1969; Frankie *et al* 1974; Gentry 1974; Opler *et al* 1980; Bawa 1983). Despite slight variation in the onset and termination of flowering of individual trees, both the seasons are compact.

Of the different visitor species (table 2), the carpenter bees (*X. latipes* and *X. pubescens*) were found to be the most appropriate and reliable pollinators of

Table 2. Flower visitors on *M. oleifera*, their forage type, occurrence at different study sites in 1983 and 1984.

Name of forager	Forage type	Siripuram				Isakathota				II Town police station				BVK College			
		Feb.–May 1983	Feb.–May 1984	Sep.–Nov. 1983	Sep.–Nov. 1984	Feb.–May 1983	Feb.–May 1984	Sep.–Nov. 1983	Sep.–Nov. 1984	Feb.–May 1983	Feb.–May 1984	Sep.–Nov. 1983	Sep.–Nov. 1984	Feb.–May 1983	Feb.–May 1984	Sep.–May 1983	Sep.–May 1984
Hymenoptera																	
Apidae																	
Apis florea	Nectar	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
A. cerana indica	"	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trigona sp.	"	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Xylocopidae																	
Xylocopa latipes	"	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
X. pubescens	"	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Anthophoridae																	
Amegilla sp.	"	–	+	+	+	–	–	–	–	–	–	–	–	–	+	–	–
Eumenidae																	
Delta pyriformis	"	–	–	–	–	+	–	+	–	–	–	–	–	–	–	+	–
Vespidae																	
Ropalidia spathulata	"	–	+	–	–	+	+	+	+	–	–	–	–	–	–	–	+
Formicidae																	

Taxon																
Camponotus sp.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Diptera																
Muscidae																
Musca domestica	+	−	+	−	−	−	−	−	+	+	+	+	+	−	+	−
Lepidoptera																
Danaidae																
Danaus chrysippus	+	−	−	+	+	+	+	+	−	−	−	+	−	−	+	−
Pieridae																
Catopsilia pyranthe	−	+·	+	−	−	+	+	+	+	+	+	+	+	+	+	−
Papilionidae																
Papilio polytes	+	+	−	−	+	+	−	+	−	+	−	−	−	+	−	+
Hesperiidae																
Barbo bevanie	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nymphalidae																
Eurema hecabe	−	+	+	+	+	+	+	+	+	+	−	+	+	+	+	−
Phalanta phalantha	−	+	−	−	−	−	−	−	−	+	+	+	−	−	−	−
Sphingidae																
Macroglossum gyrans	+	−	+	+	+	+	+	+	−	−	−	−	−	+	+	+

+, Denotes presence; −, denotes absence.

Table 3. Number of visits per minute and the length of a visit (seconds) at *M. oleifera.*

Visitor species	Number of visits				Length of a visit			
	n	R	$\bar{x}$	SD	n	R	$\bar{x}$	SD
Apis florea	10	4–8	6	1·5	10	5–6	5·8	0·4
A. cerana indica	10	3–6	4	1·09	10	3–5	3·3	0·6
Trigona sp.	10	4–8	6	1·4	10	3–5	3·5	0·8
Xylocopa latipes	10	10–15	12	1·6	10	3–4	3·2	0·4
X. pubescens	10	10–15	11	1·5	10	3–4	3·0	0·4
Amegilla sp.	10	6–10	7	1·2	10	3–4	3·8	0·4
Danaus chrysippus	10	2–7	5	1·4	10	9–12	10·4	0·9
Catopsilia pyranthe	10	2–7	5	1·6	10	5–7	5·8	1·1
Papilio polytes	10	3–5	5	2·2	10	5–8	5·8	1·1
Barbo bevani	10	1–2	1	2·8	10	105–115	102	6·0
Eurema hecabe	10	5–12	6·5	2·3	10	6–8	6·9	0·7
Macroglossum gyrans	10	11–25	18	4·7	10	1–3	1·8	0·6

n, No. of flowers observed; R, range; $\bar{x}$, mean; SD, standard deviation.

M. oleifera. A close harmony exists between the horizontally oriented, zygomorphic (bilateral) flowers of *M. oleifera*, with a provision for alighting place in the form of lower petals, nectar concealed and accumulated in a tube of 4 mm and the probosocis length (10 mm) and the bilateral symmetry of the carpenter bee which takes only a single position i.e. nototriby, with the consequent deposition of pollen on the back of its head and/or thorax. This mode of pollen deposition is known for efficacy and economy in the utilisation of pollen (Faegri and Pijl 1979).

Breeding experiments demonstrated that *M. oleifera* is adapted both for geitonogamy and xenogamy, with larger fruit set, seed set and fecundity in the latter mode. *Xylocopa* visitation may result in either of these two modes of reproduction. However, the compact flowering seasons, steady state flowering (Gentry 1974), and the characteristic behaviour of *Xylocopa* visiting a few flowers in a bout and flying over long distances (Frankie 1976) are likely to promote more of xenogamous pollination and the attendant genetic variability. Furthermore, the carpenter bee was well distributed at all the sites studied (table 2), and cursory observations also indicated that in all the places where this plant taxon was present there was the carpenter bee.

According to Pijl (1954, 1960a, b), tropical carpenter bees (*Xylocopa*) are less oligolectic than those of the sub-tropics. However, this genus possesses an odour which is temporarily transferred to the visited flowers and greatly increases pollinator efficiency by discouraging overfrequent visits. Individuals are thus more effective pollinators though their number is normally small. Furthermore, though polytropic, they have definite species-preferences and tend to visit only one species of flower if that species is relatively abundant. The bee has an inherent tendency to travel long distances, thereby contributing to interpopulation movement of pollen. The observations on *Xylocopa* in relation to pollination are in general agreement with earlier observations by Pijl (1954), Barrows (1980) and Frankie *et al* (1983). Most of them exhibit opportunistic behaviour. *Xylocopa fimbriata* and *X. gualenensis* usually exhibit trapling behaviour (Janzen 1971). Territorial behaviour of *Xylocopa* is also well known (Janzen 1964). Males of *X. Muscaria* are usually specific in their choice of nectar plants. Female *X. muscaria* and both sexes of *X. barbatella* are

general in their choice of nectar plants. *X. latipes* and *X. pubescens* of the present study appear to be also general in their choice of nectar plant species in the biotope of the study area.

Amegilla sp. may also be treated as efficient as carpenter bees in performing pollination of the zygomorphic flowers, but their availability at all the places where *M. oleifera* is distributed is uncertain (table 2). The body size of the insect is a little smaller than that of the carpenter bee and consequently goes deeper with 3/4 of its body entering the corolla. Pollen is deposited on the back of thorax when it is seeking nectar. It appears that it is more versatile in utilising floral resources and in mediating pollen transfer in certain zygomorphic flowers of this locality.

Other bee species encountered, because of their small body size, avoid contact with anthers and stigma in their act of foraging for nectar and hence are mere visitors. The butterflies and the hawkmoth are also to be taken as mere visitors, because their proboscides do not make any contact with anthers and stigma while they take nectar. The foraging by these visitors forces the pollinators to make a larger number of visits in order to satisfy their requirement. Any adaptation that forces a pollinator to visit increased numbers of flowers should be selectively advantageous (Cruden *et al* 1983).

Sunbird visitation to the flowers of *M. oleifera* is on record (Salim Ali 1932). Cursory observations at other places like Hyderabad and Ongole in Andhra Pradesh also indicated sunbird visitation to these flowers. In such localities, the sunbirds (*Nectarinia* sp.) also serve as efficient pollinators. When they probe for nectar, pollen is deposited on the back of their beaks and is transferred to the stigma in a nototribic way. Although Krishna Raju (1985) listed *Nectarinia zeylanica* and *N. asiatica* as occurring in the first quarter of the present century at Visakhapatnam, now they are totally absent.

Acknowledgement

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Morphology of the flower and fruit of *Hydrocera triflora* Wight and Arn. emend Venkat. and Dutt—an elucidation

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Abstract. The details on the floral morphology and development of fruit and seed in *Hydrocera triflora* that escaped the attention of earlier investigators have been highlighted. The flower in *Hydrocera* is interpreted as primitive over *Impatiens*. Though *Hydrocera* resembles *Impatiens* closely, it possesses certain distinctive features on the basis of which it is suggested that *Hydrocera* be treated as a tribe, Hydrocereae or as a sub-family, Hydroceroideae under the family, Balsaminaceae.

Keywords. *Hydrocera triflora*; morphology; flower; fruit.

1. Introduction

The monotypic *Hydrocera* and *Impatiens* with about 900 species (Grey-Wilson 1980) constitute the family Balsaminaceae. Three other genera, viz., *Impatientella* Peer., *Petalonema* Peter non Correns and *Semeiocardium* Zoll. were earlier included in the family. Grey-Wilson (1980), however, considers these 3 genera as congeneric with *Impatiens*. While *Hydrocera* is restricted to Indomalayan region, the species of *Impatiens* are distributed in tropical and sub-tropical regions of the old world and also occur in temperate regions of north America, Europe and Asia.

The embryology and floral anatomy of *Hydrocera* and a few species of *Impatiens* have been studied (Venkateswarlu and Lakshminarayana 1957; Narayana 1963, 1965, 1974). More recently, Grey-Wilson (1980) made certain observations on the floral morphology of *Hydrocera* and discussed its affinities with *Impatiens*. His observations on perianth and androecium broadly agree with those of Venkateswarlu and Dutt (1961) and Narayana (1974) while those on fruit and seed are at variance with the earlier reports of Venkateswarlu and Lakshminarayana (1957) and Venkateswarlu and Dutt (1961). The present study was therefore undertaken to critically examine the differences in the observations of Grey-Wilson (1980) and others (Venkateswarlu and Lakshminarayana 1957; Venkateswarlu and Dutt 1961; Narayana 1974) and to give a correct account of the details of development of fruit wall and seed coat that escaped the attention of the earlier investigators.

2. Materials and methods

Flowers and fruits of different stages of development were collected at Sarpavaram, a place near Kakinada, Andhra Pradesh and were fixed in FAA. The material was processed and embedded in paraffin wax following conventional methods. Sections were cut at a thickness of 10–14 μm and were stained using crystal violet and erythrosin combination.

3. Results

The hypogynous bracteate flower of *Hydrocera* is tetracyclic, pentamerous and zygomorphic owing to the development of a spur on the posterior sepal. The perianth parts are free and their traces also arise independently from the main stele. The stamens exhibit union by filaments just below the anthers which surround the top of the ovary as reported earlier by Narayana (1974).

The gynoecium is 5-carpellary, syncarpous, 5-locular and the placentation is axile (figure 1). The 3 ovules in each loculus are suspended from a 3-lobed placenta (figure 4). Though the gynoecium is predominantly 5-carpellary and 5-locular, 6-carpellary gynoecia with as many locules (figure 2) and 6-carpellary with only 5 locules have also been encountered for the first time (figure 3). In transections of the 6-carpellary and 5-locular ovary, one of the locules showed 6 ovules and another only two (figure 3) because of one ovule which is located at a different level did not come into view. Of the 3 ovules in each locules, one is dorsal and the other two are lateral (figures 1, 2). The ovules abut against one another by their inner surfaces and are free from the wall of the loculus (figure 5). In the regions of contact the epidermal layers of the abutting ovules are distinct (figure 6).

At this stage the ovary wall shows the outer and inner epidermal layers and 8–9 layers of intervening cells which are thin walled with prominent intercellular spaces (figure 7). The cells of the inner epidermal layer and the subepidermal layer are tangentially elongated (figure 7). The layer of cells below the outer epidermis and some of the intervening cells show accumulation of dark staining contents (figure 7).

After fertilization the gynoecium develops into the fruit. The number of layers in the pericarp remain the same but the cells enlarge considerably and the intercellular spaces become larger (figure 8). The walls of some of the parenchyamatous cells of

Figures 1–18. *H. triflora.* **1.** TS 5-carpellary, 5-locular ovary with 3 ovules in each loculus. **2.** TS 6-carpellary, 6-locular ovary with 3 ovules in each loculus. **3.** TS 6-carpellary, 5-locular ovary with 6 ovules in one loculus and 2 in another and 3 in each of the other 3 loculi. **4.** TS ovary showing 3-lobed placenta and ventral bundles opposite the loculi. **5.** TS ovary-one locule enlarged showing 3 abutting ovules. **6.** Same as in figure 5 showing intact epidermal layers of the abutting ovules. **7.** TS ovary wall. **8.** TS fruit wall. Note large air spaces and intercellular spaces. **9.** TS locule of young fruit enlarged. Note enlarging seed. **10.** Same as in figure 9 at an advanced stage. **11.** Mature seed showing the adpressed remnants of abortive seeds on the inner side. **12.** TS mature seed showing seed coat, a thin layer of endosperm and cotyledons. **13.** TS part of young seed showing development of seed coat from epidermal cells of the outer integument. Note enlarging epidermal cells opposite the funicular vascular bundle and on the antiraphe side. **14.** TS part of abortive seed on the raphe side. Note limited number of cells of the seed coat and the thin walled cells opposite the funicular vascular bundle. **15.** TS part of the developing seed on the raphe side showing thick walled cells of seed coat and elongating thin walled cells opposite the funicular vascular bundle. **16.** Side view of the mature seed showing the ridges and furrow in the seed coat and remnants of the degenerating seed appressed to it. **17.** TS mature seed coat in the region of the ridge on the antiraphe side. Note elongated thin walled cells in the seed coat. **18.** TS mature seed coat in the region between the ridges.

(ep, Epidermal layers of abutting ovules; as, air space; ics, intercellular space; ras, remnants of abortive seeds; sc, seed coat; twc, thin walled cells, fb, funicular vascular bundle; rf, ridges and furrows).
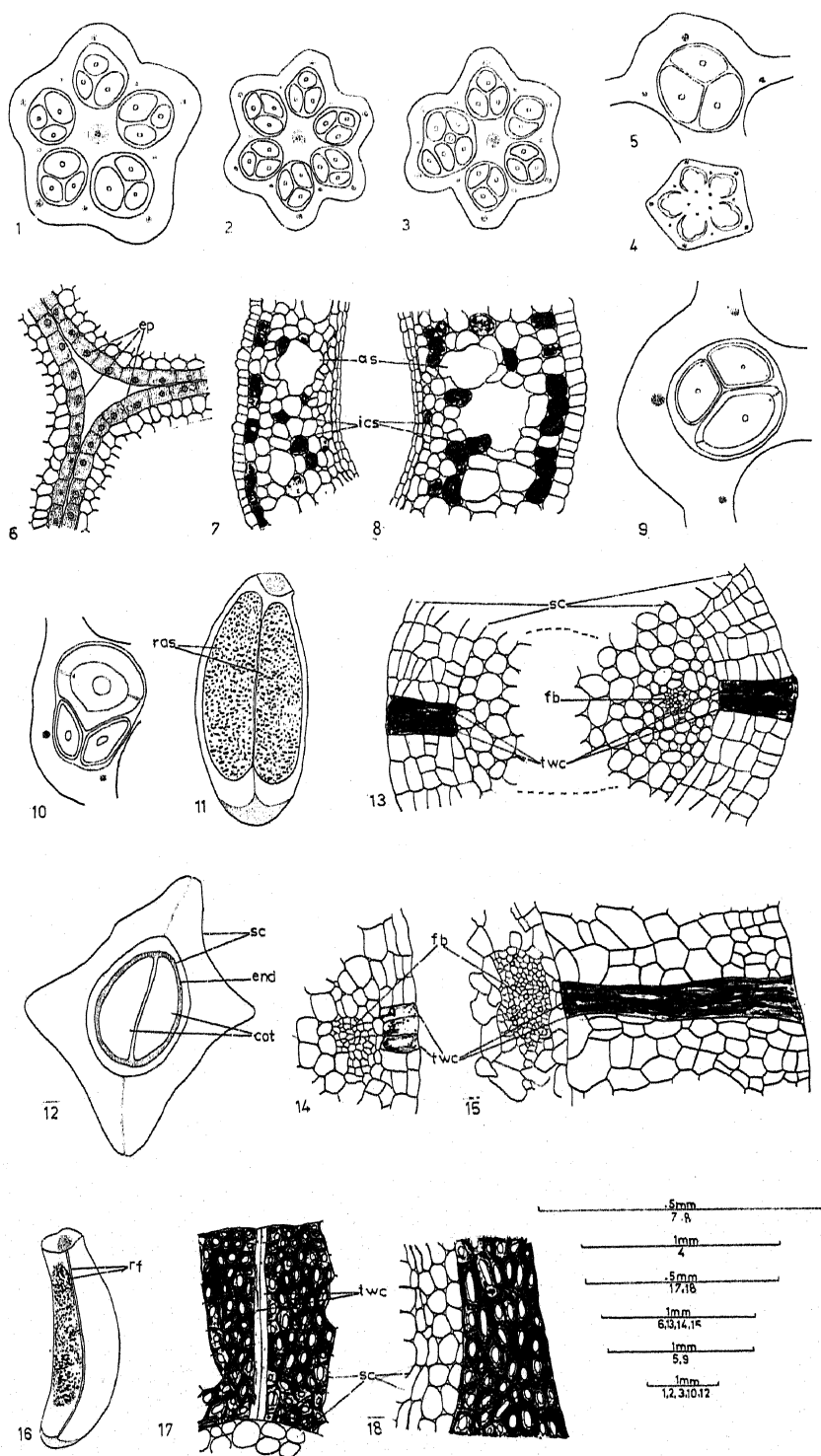
Figures 1–18.

the pericarp break down leading to the formation of large air cavities which help the ripe fruits float on water (figure 8). The cells of the ovary wall in no stage of the development of the fruit show any special alignment of the cells so common in dehiscent fruits. Thus, the anatomy of the fruit wall does not give a clue to its dehiscent nature.

All the 3 ovules develop normally till the mature embryo sac stage and for a limited time after fertilization but subsequently two of them, the one towards the dorsal side and one of the two lateral ovules, abort (figures 9, 10). The other lateral ovule alone develops into the seed (figures 9, 10). The remnants of the abortive seeds persist and remain adpressed to the mature seed on its inner side (figure 11).

The mature seed is prominently 4-angled, along the raphe and antiraphe sides and at right angles to them (figure 12). The seed coat is derived from the outer epidermis of the outer integument. These epidermal cells undergo repeated periclinal divisions cutting off cells to the outerside (figure 13). In the abortive seeds also the epidermal cells divide periclinally and the derivatives which remain only 2–4 layered, remain thin walled (figure 14). In the fertile seeds 2–4 epidermal cells opposite the funicular vascular bundle and on the antiraphe side do not divide periclinally but remain thin walled and elongate keeping pace with the divisions in the surrounding cells (figures 13, 15). This feature is also observed in the abortive seeds (figure 14).

A critical examination of the surface of the mature seed revealed two ridges and a furrow along each of the raphe and antiraphe sides. The former corresponds to the margins of the seed coat and the latter to the region of the elongated thin walled cells of the seed coat (figure 16).

The seed coat is 10–13 cells thick in the region of the ridges (figure 17) while in the other regions it is 7–9 cells thick (figure 18).

As the seed develops the embryo sac enlarges and encroaches upon the thin walled cells of the fused integuments leaving only a few layers of cells in the mature seed. Below these layers of cells a layer of endosperm cells persists in the mature seed (figure 12).

4. Discussion

In the basic 5-merous and 4-cyclic floral plan *Hydrocera* resembles *Impatiens* (Narayana 1974; Venkateswarlu and Dutt 1961; Rama Devi and Narayana 1989) but differs from *Impatiens* in the freedom of perianth parts and independent origin of their traces and can be reckoned as more primitive than *Impatiens*.

According to Grey-Wilson (1980), the anterolateral sepals in *Impatiens* have disappeared during the evolution of the flower. They are, however, inconspicuous in most species but conspicuous in *I. hongkongensis*, *I. tinctoria* and *I. quadrisepala* (Grey-Wilson 1980). There is thus a gradual tendency towards suppression of the anterolateral sepals. The reported loss of anterolateral sepals is due to adnation between the anterior petal and anterolateral sepals resulting in the formation of a compound structure on the anterior side. The origin and branching of the trace that supplies the composite structure supports this contention (Narayana 1974; Rama Devi and Narayana 1989).

All the petals are free in *Hydrocera* whereas in *Impatiens* 4 of the 5 petals unite to form two lateral pairs and the fifth one is incorporated into the anteriorly situated

composite perianth part (Grey-Wilson 1980; Narayana 1974; Rama Devi and Narayana 1989). *Hydrocera* and *Impatiens* resemble each other in their androecial character.

The observations of Grey-Wilson (1980) on the ovary and fruit of *Hydrocera* are at variance with those of the earlier investigators (Venkateswarlu and Lakshminarayana 1957; Venkateswarlu and Dutt 1961) and of the present authors. In the 5-carpellary, 5-locular ovary, *Hydrocera* resembles *Impatiens*. However, in having the 5-traced carpels and only 3 ovules being suspended from a common 3-lobed placenta, *Hydrocera* differs from *Impatiens* in which the carpels are 3-traced and the number of ovules varies from few to many in each locules (Narayana 1974; present study). Therefore the statement of Cronquist (1981) that the locules are uniovulate in *Hydrocera* appears to be incorrect. However, 6-carpellary ovaries with 6 or 5 locules have been observed in some flowers of *Hydrocera* for the first time. In the latter situation one locule showed 6 ovules suggesting the fusion of two loculi of the adjacent carpels. It may thus be inferred that the 5-carpellary condition in *Hydrocera* may have been derived from a multicarpellary ancestry.

Grey-Wilson's (1980) report that each loculus in *Hydrocera* has 3 closely adhering compartments of which only one bears the ovule the others remain empty, is certainly incorrect. The present study and earlier reports clearly show that each loculus in *Hydrocera* has 3 ovules, of which only one matures into a seed. However, the remnants of the abortive seeds persist and lie appressed to the mature seed on the inner side.

The fruit of *Impatiens* is a capsule while in *Hydrocera* it has been variously described as a drupe (Bentham and Hooker 1862–1893), a 5-seeded stone (Dunn 1967), a capsular berry (Venkateswarlu and Dutt 1961), a 5-seeded indehiscent berry (Grey-Wilson 1980), a fleshy pseudoberry with a pentagonal outline (Grey-Wilson 1980) and a pentagonal, berry-like drupe with the stone separating into five 1-seeded pyrenes (Cronquist 1981).

The present critical study on the development and structure of the fruit wall and seed coat in *Hydrocera* casts a doubt as to the correctness of the reports of earlier investigators (Venkateswarlu and Dutt 1961; Grey-Wilson 1980). The observations of Grey-Wilson (1980) that the common wall surrounding the 3 compartments of each carpel 'becomes part of the endocarp which is embedded in the soft tissues of the surrounding mesocarp' are contrary to facts. The present study clearly shows that the fruit wall does not at any stage differentiate into the epicarp, mesocarp and endocarp. The thick walled cells of the seed coat of the maturing seed and the few layers of the cells of the seed coat of the abortive seeds become closely appressed to the loculus giving a false impression of endocarp. Therefore, the description of the fruit as a drupe by Venkateswarlu and Lakshminarayana (1957) and a berry like drupe by Cronquist (1981) is untenable.

The statement of Grey-Wilson (1980) that each seed in *Hydrocera* is associated with two air sacs derived from the empty compartments of a loculus is also erroneous. The present study and observations of Venkateswarlu and Lakshminarayana (1957) and Venkateswarlu and Dutt (1961) clearly show that what have been mistakenly interpreted as 'air sacs' are nothing but the remnants of the persisting abortive seeds adpressed to the mature seeds.

The observations of the present authors on the dispersal of fruits in *Hydrocera* are at variance with those of Grey-Wilson (1980) according to whom the ripe fruits

are heavy and hence sink after they fall into the water. After the decay of the soft tissues of the fruit wall the endocarp separates into 5 units each consisting of a thick walled seed coat and two hollow compartments which function as air sacs (Grey-Wilson 1980). The observations of the authors clearly show that the ripe fruits fall into the water and float on the surface of water and are thus dispersed by water currents but do not sink due to the weight of the fruit as reported by Grey-Wilson (1980). The intercellular spaces and the large air spaces that develop due to the dissolution of the walls of some of the parenchymatous cells of the pericarp lend buoyancy for the fruit to float on water. As the water in the puddles dries up the fruits settle at the bottom and lie there till the next season. By then the pericarp decays and the 5 stony seeds which are set free, then germinate in the next season. The statement of Cronquist (1981) that the endocarp (stone) separates into five 1-seeded pyrenes is untenable because the pericarp does not show any differentiation of endocarp. The stony seeds are separated from one another by the septa and separate from one another after the disintegration of the fruit wall and not due to breaking up of the endocarp as reported by Cronquist (1981).

Any characteristic alignment of the cells in the fruit wall generally met with in the dehiscent type of fruits, is absent in *Hydrocera* and this clearly rules out the dehiscent nature of the fruit. In the absence of any external and anatomical evidence for the dehiscence, the fruit in *Hydrocera* can be described as a berry with 5 stony seeds.

The seed coat in *Hydrocera* is formed entirely from the cells formed by the periclinal divisions in the cells of the outer epidermis of the outer integument. However, a few layers of thin walled cells of the inner integument and a layer of endosperm cells also persist (Venkateswarlu and Lakshminarayana 1957; present study). In *Impatiens* the seed coat is formed by the outer epidermis of the outer integument and a few layers below it (Narayana 1965). Grey-Wilson (1980) reported many types of 'processes' on the testa of *Impatiens* species examined by him and attributed functions of anchorage, uptake of water and protection against desiccation.

Another noteworthy feature which escaped the attention of earlier investigators is the discontinuities in the seed coat along the region external to the funicular vascular bundle and the corresponding opposite side, formed by the elongation of 2–4 epidermal cells instead of their division as in the adjacent cells. These discontinuities appear as furrows between two ridges corresponding to the margins of the seed coat along the raphe and antiraphe sides. These furrows may be concerned in the diffusion of water necessary for the germination of the seed.

From the above discussion it is obvious that the genus *Hydrocera* though resembling *Impatiens* in essential floral morphological characters, differs from it in the semiaquatic habitat, 3-flowered inflorescence, absence of connation between the perianth parts and their traces, 5–6 carpellary ovary, 5-traced carpels, 3 ovules per locule suspended from a 3-lobed placenta, indehiscent fruit (berry with 5 stony seeds) and the seed coat of thick walled cells derived from the outer epidermis of the outer integument. In view of these significant differences between *Hydrocera* and *Impatiens* it is tentatively suggested that *Hydrocera* perhaps deserves to be treated either as a sub-family, Hydroceroideae or as a tribe Hydrocereae, under the family Balsaminaceae.

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An embryological approach to the taxonomical status of *Hedyotis* Linn.

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Abstract. Structure and development of male and female gametophytes, endosperm, embryo, seed coat and fruit wall are described for 8 species of *Hedyotis*. Based on morphological and embryological features the systematic position of the genus is discussed.

Keywords. Embryology; taxonomy; *Hedyotis*; Rubiaceae.

1. Introduction

Hedyotis Linn. (*Oldenlandia* Linn.) the herbaceous genus, belongs to the tribe Oldenlandieae K Schum. (= Hedyotideae C and S and DC) of the subfamily Cinchonoideae of Rubiaceae. It comprises about 400 species (Lewis 1962) and is distributed in all continents except Europe. A few species are therapeutic.

Due to the polymorphous nature of the genus, diverse opinions are expressed by different taxonomists with regard to its systematic delimitation. Therefore, in the present study, an attempt has been made to discuss its systematics based on embryological features. As many as 8 species of *Hedyotis*—*Hedyotis* (*Diplophragma*) *stylosa* R Br. (= *Oldenlandia stylosa* O Kze); *H.* (*Anotis*) *quadrilocularis* ThW. (= *Anotis quadrilocularis* Benth.); *H. auricularia* Linn. (= *O. auricularia* K Schum. = *Exallage auricularia* (Linn.) Bremek.); *H.* (*Oldenlandia*) *alata* W and A (= *O. alata* Koen), *H.* (*O.*) *biflora* (Linn.) W and A (= *O. biflora* Linn.), *H.* (*O.*) *herbacea* Linn.; *H.* (*O.*) *aspera* Heyne ex Roth and *H.* (*O.*) *gracilis* Hook. f. (= *K. gracilis* (Wall ex Roxb.) DC) have been investigated.

The early embryological studies are those of Lloyd (1902), Fagerlind (1937), Raghavan and Rangaswamy (1941), Farooq (1953, 1958), Siddique and Siddique (1965), Farooq and Inamuddin (1969), Shivaramaiah (1971), Shivaramaiah and Sundara Rajan (1973), Prakasa Rao and Sarat Babu (1975), Sivaramaiah and Sankara Rao (1977), Ahmed (1978a, b) and Narmatha Bai and Lakshmanan (1984).

2. Materials and methods

The materials of *H.* (*O.*) *aspera*, collected by Sri K Vanamala Naidu from Chittoor and the rest of the species collected by the authors at various places—*H.* (*D.*) *stylosa* at Kodaikanal, *H.* (*A.*) *quadrilocularis* at Paderu, *H. auricularia* and *H.* (*O.*) *gracilis* at Araku, *H.* (*O.*) *alata* and *H.* (*O.*) *biflora* at Kesanakurrupalem and *H.* (*O.*) *herbacea* at Visakhapatnam were fixed in FAA. Customary methods of dehydration, clearing and embedding were followed according to Johansen (1940). Sections cut at 6–14 µm were stained with Delafield's haematoxylin.

3. Results

3.1 *Flower*

The flowers are usually tetramerous and rarely trimerous with squamella inside the calyx alternating with the sepals (figure 1).

3.2 *Microsporangium, microsporogenesis and male gametophyte*

The anther is tetrasporangiate (figures 1, 9) with dicotyledonous type of wall development. It consists of an epidermis, hypodermal layer, middle layer and tapetum (figures 2–12). The secretory tapetum is uniseriate and monomorphic with uninucleate cells, but in *H. auricularia* it is biseriate at places (figure 8). The fibrous endothecium is usually uniseriate but becomes bi- or triseriate towards connective (figure 13).

The archesporium consists of a plate of 2–4 rows of cells (figure 4). The pollen mother cells undergo simultaneous cytokinesis to form tetrahedral, isobilateral, decussate or rhomboidal tetrads (figures 14–20). Although liberation of pollen grains from the tetrads is normal (figures 21–27), in a few cases of *H. auricularia*, the pollen remain united in tetrads and shed as such (figures 28–30). The pollen liberate at 2-celled stage (figures 24–26) in all except in *H. (A.) quadrilocularis* where they shed at 3-celled stage (figure 27). Normally the pollen grains are triaperturate (figures 21, 24) and rarely tetra or penta aperturate (figures 22, 25). However, they are tetra aperturate in *H. (O.) gracilis* and multiaperturate in *H. (A.) quadrilocularis* (figure 27). The exine is thick and smooth in all the species except in *H. (A.) quadrilocularis* and *H. (O.) herbacea* where it shows radial striations (figures 21, 25, 27).

H. (O.) gracilis exhibits pollen polymorphism (figure 26). Pollen degeneration at different stages of development is quite common.

3.3 *Ovary and ovule*

The ovary is inferior, bicarpellary syncarpous and bilocular (figures 31, 33). Rarely, in *H. (O.) aspera* and *H. (O.) alata* it is trilocular (figure 32). However, in *H. (A.) quadrilocularis* it is characteristically tetralocular and at times bilocular. Axile placentae bear numerous ovules.

The ovule is hemianatropous, unitegmic and tenuinucellate. The nucellus in all the species investigated is of Oldenlandia type with a single nucellar epidermal cell (figures 34, 37). However, in *H. (O.) aspera* it is of Bouvardia type with 3 nucellar epidermal cells (figure 36). Rarely, in *H. (A.) quadrilocularis, H. auricularia, H. (O.) alata* and *H. (O.) aspera* the nucellus consists of two cells (figures 35, 38).

3.4 *Megasporogenesis and female gametophyte*

The single archesporial cell directly functions as the megaspore mother cell without cutting off a parietal cell (figures 35–38). As a result of meiotic division a linear tetrad of megaspores is formed (figures 39, 40). The chalazal one of the tetrad develops into an 8-nucleate embryo sac of the Polygonum type (figures 44–54). The
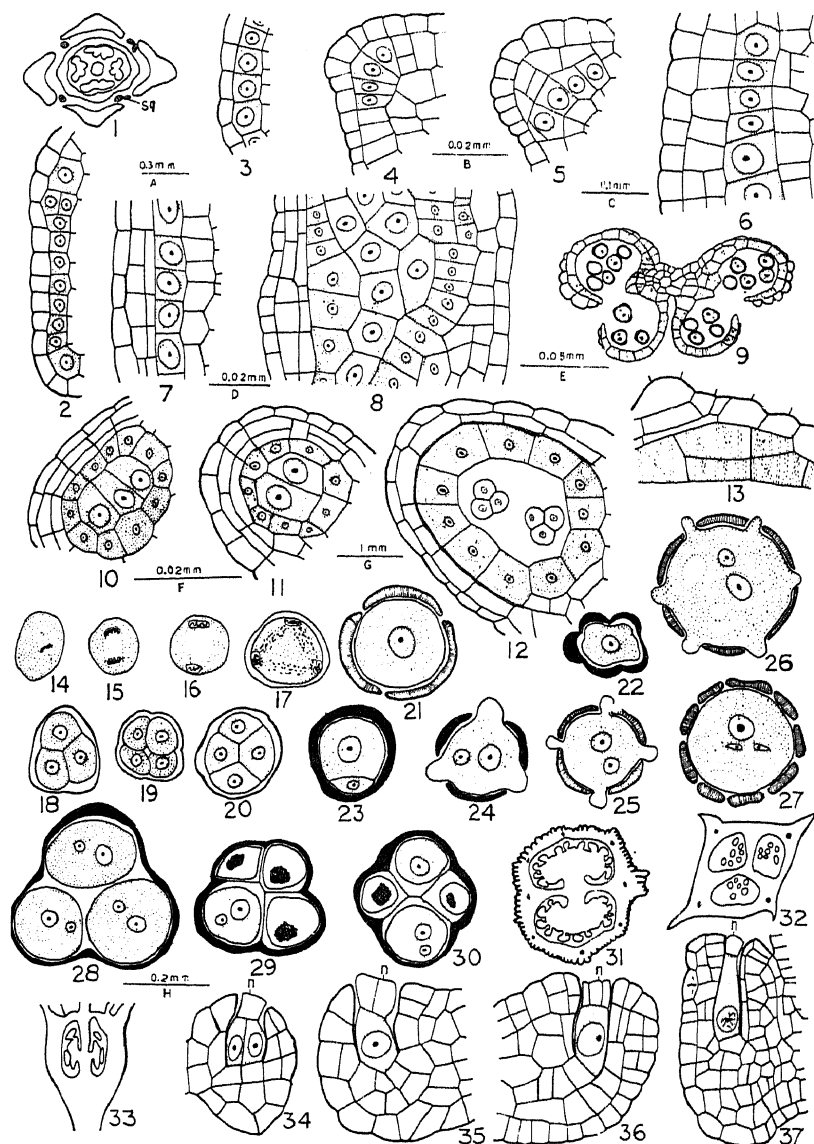
Figures 1–37. **1, 18, 21, 32.** *H. (O.) herbacea.* **2, 9, 10, 17, 20, 32, 34.** *H. (O.) alata.* **3, 11, 27.** *H. (A.) quadrilocularis.* **4, 5, 26.** *H. (O.) gracilis.* **6, 13, 37.** *H. (D.) stylosa.* **7, 18, 14, 15, 28, 30.** *H. aricularia.* **16, 23.** *H. (O.) biflora.* **12, 19, 22, 25, 31, 36.** *H. (O.) aspera.* **1.** Ts of flower showing squamella. **2–4.** Ls of part of anther lobe showing archesporium. **5 and 6.** Ls of part of anther lobe showing parietal layer and sporogenous layer. **7 and 8.** Ls of part of anther lobe showing wall layer and sporogenous tissue. **9.** Ts of dehisced anther. **10 and 11.** Ts of anther layer showing wall layer and pollen mother cell. **12.** Ts of anther layer showing pollen tetrads. **13.** Ts part of anther lobe showing two layered endothecium towards connective. **14–17.** Pollen mother cells in meiotic division. **18–20.** Pollen tetrads. **21–27.** Pollen grains. **28–30.** Pollen grains remained in tetrads. **31 and 32.** Ts of ovary. **33–37.** Ls of ovules. (sq, Squamella; n, nucellus).
(Magnification: Scale A for 1, 31, 32; B for 2–8, 10, 11, 14–16, 26–30, 35, 36; C for 9; D for 12; E for 13, 37; F for 17–25; G for 33; H for 34).
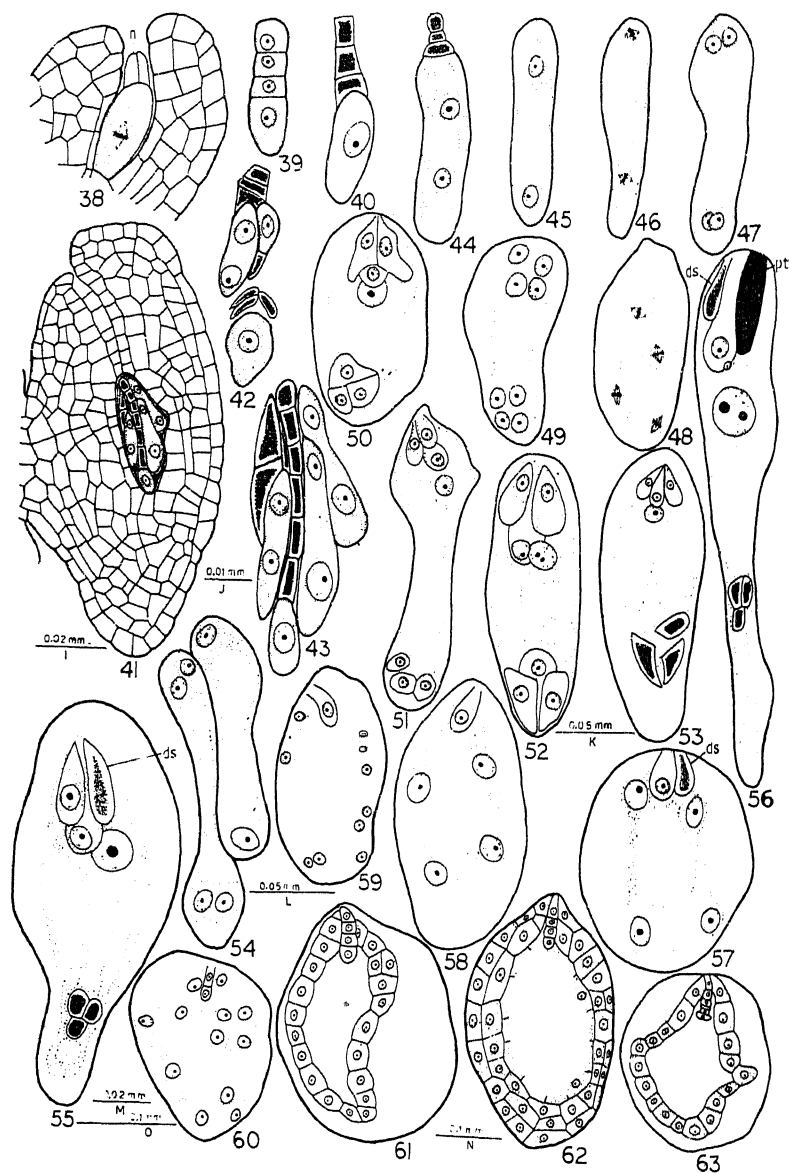
Figures 38–63. **38, 50, 59, 61.** *H.* (*A.*) *quadrilocularis.* **39, 53, 58.** *H.* (*O.*) *herbacea.* **40, 45, 47, 51, 62.** *H.* (*O.*) *aspera.* **41–43, 46, 55, 56.** *H.* (*D.*) *stylosa.* **44, 48, 49, 52.** *H. auricularia.* **54, 60, 63.** *H.* (*O.*) *biflora.* **38.** Ls part of ovule showing megaspore mother cells and two nucellar epidermal cells. **39 and 40.** Megaspore tetrads. **41.** Ls ovule showing multiple tetrads and embryo sacs. **42.** Multiple tetrads. **43.** Multiple tetrads and embryo sacs. **44–53.** Embryo sac development. **54.** Twin embryo sacs. **55.** Embryo sac with chalazal caecum. **56.** Embryo sac showing syngamy and triple fusion. **57–63.** Endosperm development. (n, Nucellus; ds, degenerating synergids; pt, pollen tube). (Magnification: Scale I for **38, 42–45, 47–52, 55, 56, 58, 59;** J for **39, 40, 53, 54;** K for **41, 61;** L for **46;** M for **57;** N for **60, 63;** O for **62**).

synergids are pear-shaped. They are hooked only in *H. (A.) quadrilocularis* (figure 50). The polars fuse near the egg apparatus. The 3 uninucleate antipodals degenerate either before or soon after fertilization.

In *H. (D.) stylosa* the chalazal end of the 8-nucleate embryo sac extends into the ovular tissue and functions as haustorium (figures 55, 56). In *H. (O.) alata* 2-celled archesporium (figure 34), in *H. (D.) stylosa* multiple tetrads and embryo sacs (figures 41–43) and in *H. (O.) biflora* twin embryo sacs (figure 54) occur occasionally.

3.5   *Fertilization, endosperm and embryo*

The pollen tube enters the embryo sac through micropyle and destroys one of the synergids (figure 55). Syngamy and triple fusion occur more or less simultaneously (figure 56).

Endosperm is *ab initio* nuclear. The primary endosperm nucleus divides earlier than the zygote to form a few free nuclei which become distributed in the periphery of the embryo sac (figures 57–60). By the time a 4-celled embryo is formed, cell wall formation commences from the periphery to the centre ultimately filling the entire embryo sac with cellular tissue (figures 61–63). Starch globules develop in the cells of the endosperm (figure 64). The seeds are endospermic (figures 83–85).

The zygote divides transversely resulting in a 2-celled proembryo (figures 65, 66). The terminal cell *ca* and the basal cell *cb* undergo each one more transverse division to form a linear 4-celled proembryo (figure 68). The cells are termed as l, l', m and *ci* from the apex to the base. Sometimes in *H. (O.) aspera* the cell *ca* divides earlier resulting in the formation of a 3-celled proembryo (figure 67). The cells m and *ci* divide transversely to form a linear 6-celled proembryo (figures 69–71). The cells l and l' divide vertically twice resulting in quadrants. Further vertical division in them leads to the formation of octants (figures 72–77). The two tiers l and l' divide transversely to form 4 tiers—l_1, l_2, l'_1 and l'_2 (figure 78).

The derivatives of l contribute to the formation of cotyledons and stem tip and those of l' to hypocotyl and root. The cells d, f, n and n' form a uniseriate suspensor of 4 cells (figures 79–81). As the 4 celled proembryo is linear and the terminal cell *ca* alone contributes to the formation of embryo proper, the embryogeny is of Solanad type. The proembryo consists of 6 cells arranged in 6 tiers at the third cell generation and this conforms to the Nicotiana variation.

3.6   *Testa*

The shape of the seed is variable. It is round in *H. (O.) biflora* (figure 82), concave in *H. (A.) quadrilocularis* (figure 84) and angular in the rest of the species (figures 83, 85). The integument at the megaspore mother cell stage is 2–5 celled thick and remains so up to the 8-nucleate stage of the embryo sac except for volumetric increase of the cells. However, in *H. (D.) stylosa* the number of wall layers increases up to 12 at organised embryo sac stage. At this stage tannin deposition occurs in all the species except in *H. (O.) aspera* where it occurs only after fertilization. As a result of fertilization the integument increases in its number of wall layers as well as size of cells. It is somewhat bulky at the globular embryo stage. At the dicot stage of the embryo, disintegration of all the wall layers occurs except the epidermis which alone forms the testa of the seed (figures 83–85). The testa is filled with tannin
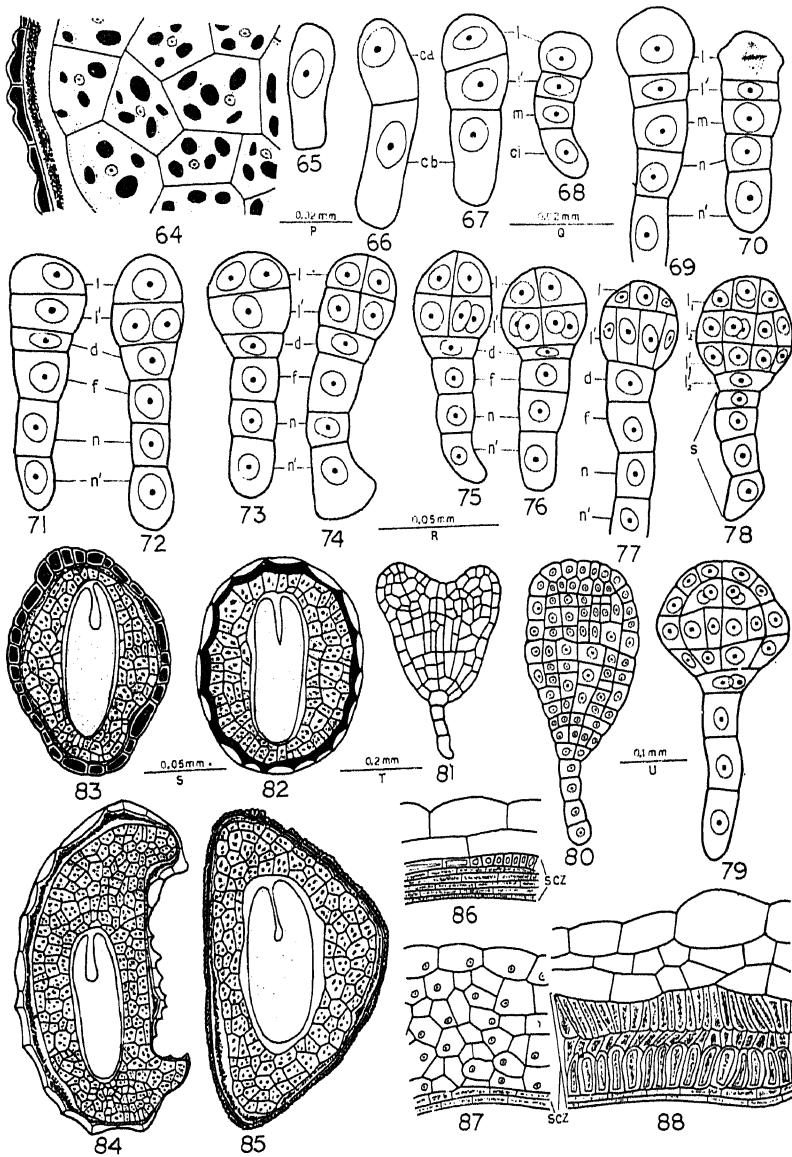
Figures 64–88.   **64, 77, 85, 86.** *H. (O.) herbacea.* **65, 67, 69, 71–74, 80, 88.** *H. (O.) aspera.* **68, 78, 79, 84.** *H. (A.) quadrilocularis.* **70, 75, 76, 81–83, 87.** *H. (O.) biflora.*   **64.** Ls of part of seed coat and cellular endosperm with storage products.   **65–81.** Embryo development. **82–85.** Section of seeds showing embryo and seed coat.   **86–88.** Fruit wall. (S, Suspensor; SCZ, sclerified zone).
(Magnification: Scale P for **64, 68, 70, 75, 76, 78, 79**; Q for **65, 69, 71–74, 77**; R for **80**; S for **81, 86, 88**; T for **82–85**; U for **87**).

except in *H. (O.) biflora* and *H. (O.) quadrilocularis* where the inner and/or tangential walls of the cells become cutinized (figures 82, 84). In *H. (O.) herbacea* the outer walls of the cells are dentate (figure 85).

3.7 *Pericarp*

At the megaspore mother cell stage, the ovary wall is 6–10 layered in all the species except in the shruby *H. (D.) stylosa* where it is 15–20 celled thick. Raphides occur in the ovary wall. The pericarp is discernible into two zones. The inner zone is 4–6 layered with compactly arranged smaller cells and the remaining wall layers with larger cells form the outer zone. After fertilization the inner zone becomes sclerified. In a mature fruit 3–6 layers of the outer zone and the entire sclerified inner zone together constitute the pericarp (figures 86–88). Here and there on the fruit wall unicellular hairs occur in *H. auricularia* while in *H. (O.) aspera* some of the epidermal cells become bulged.

4. Discussion

Taxonomically *Hedyotis* is treated variously by various taxonomists. Linnaeus (sp. pl. 1753) recognized *Hedyotis* and *Oldenlandia* as distinct genera. Brown and Wallich (c.f. Wight and Arnott 1834) clubbed the 4 different genera—*Oldenlandia, Anotis, Kohautia* and *Hedyotis* into a single genus namely *Hedyotis*. This gains support from Fosberg (1941, 1954), Shinners (1949), Lewis (1959, 1965) and Rao and Hemadri (1973). Wight and Arnott (1834) although accepted the mixed genus *Hedyotis*, divided it into 5 sections namely (i) *Diplophragma*, (ii) *Anotis*, (iii) *Euhedyotis* (= *Exallege*), (iv) *Scleromitrion* (= *Hedyotis*) and (v) *Oldenlandia*. They treated *Kohautia* as a subsection of *Oldenlandia*. On the other hand Chamisso and Von Schlechtendal (1828) treated each of the above taxa as an independent genus. Bremekamp (1952) is in favour of this treatment.

The embryological features of all the above sections except the section *Scleromitrion* together with their morphological characters are tabulated in table 1. From the table it is clear that the section *Diplophragma* differs from other sections in the shrubby habit, purple corolla, presence of chalazal embryo sac haustorium, massive wall of ovule and ovary and dicoccus fruit. Thus, based on morphological and embryological evidence the merging of *Diplophragma* into *Hedyotis* as treated by Wight and Arnott (1834) is not favoured. It may be retained as an independent genus *Diplophragma*.

The section *Anotis* differs from the rest of the taxa in the presence of multiaperturate and 3 nucleate pollen grains, tetralocular ovary, hooked synergids, crustaceous fruit, boat shaped or plano-convex seeds and absence of tannin in the testa. Therefore, the inclusion of *Anotis* in the genus *Hedyotis* as is done by Brown and Wallich (c.f. Wight and Arnott 1834) and Wight and Arnott (1834) seems to be inappropriate. On the otherhand, it appears to be justified to assign a generic rank to *Anotis* as was treated by De Candolle (1830), Bentham and Hooker (1862–1883), Schumann (1891) and Melchoir (1964). According to Lewis (in Ann. Missouri Bot. Gard. 53:38. 1966) the generic name *Anotis* DC is restricted to the new world species and is distinct from the old world species including Indian spp. for which he has proposed a new generic name, *Neanotis* W H Lewis.

From the table 1 it is evident that the remaining two sections *Euhedyotis* and *Oldenlandia* including *Kohautia* share a number of common morphological and embryological features. Hence, their merging into a single genus *Hedyotis* appears to be justified.

Table1. Morphological and embryological characters of different sections of the genus *Hedyotis*.

Character	Section: Diplophragma	Section: Anotis	Section: Euhedyotis	Sub-section: Oldenlandia	Section : Oldenlandia Sub-section: Kohautia
Habit	Shrubs	Herbs	Herbs	Herbs	Herbs
Inflorescence	Terminal or axillary cyme	Axillary or terminal cyme	Axillary cyme	Axillary cyme	Axillary cyme
Flowers	Tetramerous with purple corolla	Tetramerous with white corolla	Tetramerous with white corolla	Tetramerous with white corolla	Tetramerous with white corolla
Ovary	Bicarpellary and bilocular	Bicarpellary and bi- or quadrilocular	Bicarpellary and bilocular	Bicarpellary and bi- or trilocular	Bicarpellary and bi- or trilocular
Placentation	Axile on stalked placentae	Axile on stalked placentae	Axile on stalked placentae	Swollen axile with sessile placentae	Swollen axile with sessile placentae
Ovules number	Numerous	1-8	Numerous	Numerous	Numerous
Anther	Tetrasporangiate with 4 wall layers	Tetrasporangiate with 4 wall layers	Tetrasporangiate with 4 wall layers	Tetrasporangiate with 4 wall layers	Tetrasporangiate with 4 wall layers
Tapetum	Secretory, uniseriate with uninucleate cells	Secretory, uniseriate with uninucleate cells	Secretory, uniseriate with uninucleate cells	Secretory, uniseriate with uninucleate cells	Secretory, uniseriate with uninucleate cells
Pollen tetrads	Tetrahedral	Tetrahedral	Tetrahedral, decussate or isobilateral	Tetrahedral, decussate or isobilateral	Tetrahedral, decussate or isobilateral
Pollen grains	2-nucleate and triaperturate	2 or 3-nucleate 3-multiaperturate	2-nucleate, tri- or tetra-aperturate	2-nucleate, tri- or tetra-aperturate	2-nucleate, tetraaperturate

Nucellar epidermis	1-celled	1 or 2-celled	1 or 2-celled	1-3-celled
Embryo sac	Polygonum type showing chalazal haustorium	Polygonum type. No chalazal haustorium	Polygonum type. No chalazal haustorium	Polygonum type. No chalazal haustorium
Synergids	Not hooked	Hooked	Not hooked	Not hooked
Endosperm	—	ab initio nuclear	ab initio nuclear	ab initio nuclear
Embryogeny	—	Nicotiana variation of Soland type	Nicotiana variation of Soland type	Nicotiana variation of Soland type
Integument	10–12 layered	4–6 layered	4–6 layered	6 layered
Ovary wall	20 layered	8 layered	8 layered	8 layered
Seed	Angular	Boat shaped or planoconvex	Angular	Angular or round
Testa	—	1 layered without tannin	1 layered without tannin	1 layered with/without tannin
Fruit	Ovoid, dicoccus capsule	Crustaceous, loculicidal capsule	Spherical, 2-celled capsule	Round or slightly compressed capsule	Globose or slightly compressed capsule
Pericarp	—	4–6 layered	10–15 layered with 2 zones	8–10 layered, with 2 zones	8–10 layered, with 2 zones

Acknowledgement

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Fruit and seed structure in Araceae

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Abstract. Fruit and seed anatomy of 12 species of Araceae distributed in 11 genera has been described. Taxonomic significance of fruit and seed anatomy has been brought out.

Keywords. Fruit; seed; anatomy; Araceae.

1. Introduction

Our knowledge of fruit and seed structure in Araceae is quite meagre (Netolitzky 1926). Windle (1889) recorded the occurrence of fibres (trichosclereids) and raphides in the fruits of *Monstera*. Buell (1935) stated that the seeds of *Acorus* are ensheathed by hard transparent mucilage that rapidly absorbs moisture and swells into gelatinous mass when wetted. The present study, mainly aimed at recognising taxonomic significance, describes anatomy of mature fruits and seeds of 12 species of 11 genera in Araceae.

2. Materials and methods

Mature fruits and seeds of *Pistia stratiotes* and *Aglaonema commutatum* were collected from plants cultivated in gardens of Ruia College, Bombay and those of other species from forests of various places in Maharashtra and fixed in FAA. Customary procedure of dehydration, embedding and sectioning was followed. The microtome sections of 20–30 μm thickness were stained with 1% safranin in 70% alcohol and 0.5% aniline blue in absolute alcohol.

3. Results

Fruits in Araceae are raphidian berries. Amongst the species examined, they are of composite type in *Cryptocoryne* and simple in others. The simple ones are all unilocular. The pericarp is thin and parenchymatous. It includes raphide sacs, vascular strands devoid of fibrous sheath and, occasionally the pigmented/tannin cells and druses.

The seeds are ribbed/nonribbed. The testa is massive with compactly arranged parenchymatous cells containing raphide sacs, some times pigmented cells, rarely sclerotic elements. The tegmen is thin and tanniniferous or is reduced to a layer of cuticle. The endosperm is starchy with outermost aleurone layer. Embryo is well developed, rarely adventitious and the endosperm like tissue containing raphide sacs.

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Subfamily: Lasioideae

Tribe: Pythonieae

Amorphophallus commutatus Engler

Fruit: Ovoid, one seeded; stigmatic remains apical; seed coat buff coloured.
    Fruit wall 10–12 layered in width (figure 9). Outer and inner epidermal cells rectangular, tangentially arranged with a thin layer of cuticle on the outer walls. Ground tissue of polygonal to oval, radially elongated, loosely arranged cells. Raphide sacs few. Vascular strands in a single ring (figure 13) situated in the inner part of fruit wall.

Seed: Seed coat epidermis highly cutinised, followed by 3–4 layers of polygonal, some pigmented cells; innermost layer phellogen like (figure 9). The ground tissue of polygonal cells with simple circular starch grains and conspicuous, circular raphide sacs (figure 9). Adventitious shoot bud well differentiated with lateral roots.

Subfamily: Philodendroideae

Tribe: Aglaonemateae

Aglaonema commutatum Schott

Fruit: Ovoid, one seeded, stigmatic remains, terminal. Seeds ovoid, seed coat buff coloured.
    Fruit wall 15–20 layered. Outer and inner epidermal cells tubular to rectangular, tangentially oriented, cutinised with a layer of cuticle on outer face. Ground tissue cells oval to polygonal (figure 12). Raphide sacs many, of similar size as ground parenchyma cells. Vascular strands in a ring, situated towards inner side of fruit wall (figure 12).

Seed: Seed coat with highly cutinised epidermal cells followed by 4–5 rows of phellogen like layers (figure 12). Ground tissue cells polygonal, densely filled with simple circular starch grains; raphide sacs common. Adventitious shoot bud well developed with lateral roots.

Subfamily: Colocasioideae

Tribe: Colocasieae

Subtribe: Steudnerinae

Gonatanthus sarmentosus Klotzsch

Fruit: Small, many seeded, irregularly lobed, closely arranged around the fruiting axis.
    Fruit wall 10–12 layered. Epidermal cells tubular to rectangular, tangentially oriented with cutinised walls and a layer of cuticle on outer face. Ground tissue cells polygonal, densely filled with starch grains (figure 10). Raphide sacs occasional, large, circular. Vascular strands inconspicuous.

Seed (figure 10):   Testa compressed, 4–5 layered, wider around micropyle, cells rectangular thin walled; raphide sacs in a ring, crowded in micropylar area. Tegmen two layered, cells rectangular, narrow, highly sclerified, filled with brown content.

Endosperm with peripheral layer of aleurone cells, filled with dense cytoplasm having granular contents; inner cells polygonal, thin walled, densely filled with simple starch grains having excentric hilum; embryo well developed.

Tribe: Ariopsieae

Ariopsis peltata Nimmo

Fruit: Berries sessile, partly adnate to the inflorescence axis, oblong, ±angled, unilocular, many seeded. Seeds elongated, strongly longitudinally ribbed (figure 1); ribs supported by multilayered sclerotic cells. Endosperm starchy, embryo apical.

Fruit wall 7–12 layered in width (figures 1, 2). Epidermal cells outer tubular, inner oval, tangentially arranged, with a thin layer of cuticle. Ground tissue of oval-circular cells; inner-most 2–3 layers densely filled with starch grains. Vascular strands few, restricted to inner part of fruit wall (figure 2).

Raphide sacs frequent in outer part of fruit wall.

Seed: Testa massive, lobed, each lobe internally supported by regularly arranged longitudinal sclerotic ribs alternating with parenchymatous tissue (figure 2). Outer epidermis wavy, of squarish cells; inner epidermis highly cutinised of rectangular cells. Ground tissue reduced, with a ring of oval to circular raphide sacs situated towards inner side. The ribs appear to be continuous in basal funicle also. Tegmen reduced, of 1–2 rows of radially compressed, tangentially arranged rectangular cells filled with coloured material, with thick uniform layer of cuticle on outer face (figures 1, 2). Endosperm starchy, cells polygonal, starch grains simple, circular. Embryo well developed.

Subfamily: Aroideae

Tribe: Areae

Subtribe: Arinae

Sauromatum pedatum (Willd) Schott

Fruit: Fruitlets densely arranged on the inflorescence axis, with flattened apical surface having persistant stigmatic residue in center, lateral sides 3–5-gonous; seeds 1–3 (figure 14), mostly one, erect, elongated; seed coat with starch filled testa, and brown tegmen (figure 11), embryo opical.

Fruit wall: Epidermis of cutinised cells; outer epidermal cells polygonal in surface view, rectangular in transectional view; inner epidermal cells hexagonal and longitudinally oriented in surface view, rectangular in transection and tangentially extended. Vascular strands small, in a ring towards inner side of fruit wall (figure 14). Ground tissue cells with numerous starch grains. Raphide sacs numerous, polygonal to circular.

Seed: Testa and tegmen free right to the base; testa broader at base and at micropylar apex, 8–10 layered in the middle, outer epidermal cells enlarged, tubular to rectangular, free of starch; inner epidermal cells small, squarish. Ground tissue cells hexagonal, longitudinally extended, filled with starch grains (figure 11); raphide sacs ovoid, common specially at base and apex (figure 14). Tegmen closely adhering the seed surface, 1–2 layered (figure 11) but broader at micropylar and chalazal
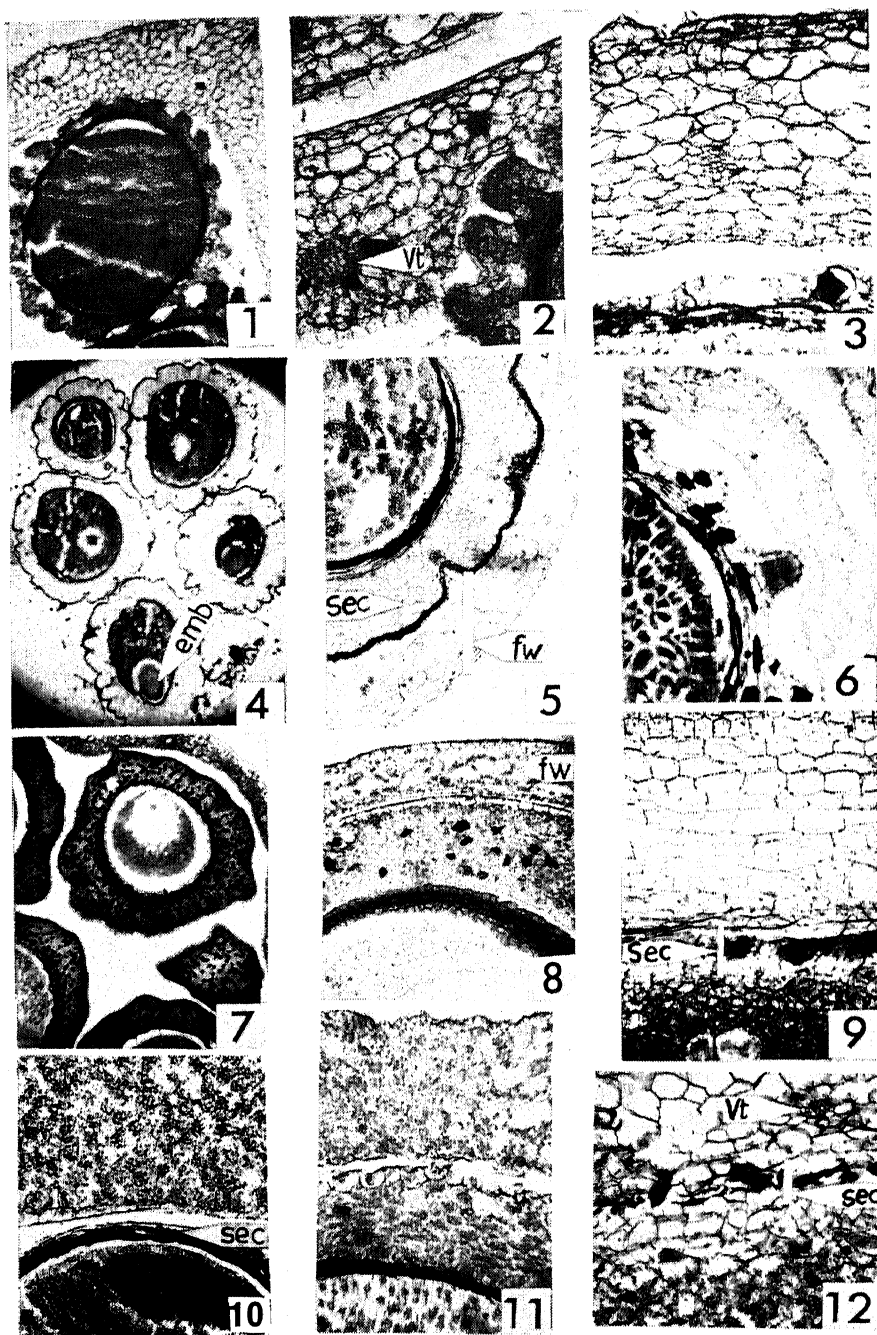
Figures 1–12. TS of fruit wall and seed. **1 and 2.** *Ariopsis peltata*. **1.** Fruit wall and seed (×150). **2.** Sector enlarged (×300). **3.** *Cryptocoryne spiralis*. Sector of fruit (×300). **4 and 5.** *Pistia stratiotes*. **4.** Gross view (×75). **5.** Sector of the fruit (×300). **6.** *Lagenandra ovata*. Sector of fruit (×300). **7.** *Theriophonum indicum*. Fruit wall along with seed (×75). **8.** *Arisaema tortuosum*. Sector of fruit (×300). **9.** *Amorphophallus commutatus*. Fruit sector (×300). **10.** *Gonatanthus sarmentosus*. Fruit wall and seed (×300). **11.** *Sauromatum pedatum*. Fruit wall and seed (×150). **12.** *Aglaonema commutatum*. Inner part of fruit wall and seed (×300).

(Vt, Vascular strands; emb, embryo; Sec, seed coat; d, druses; Fw, fruit wall).
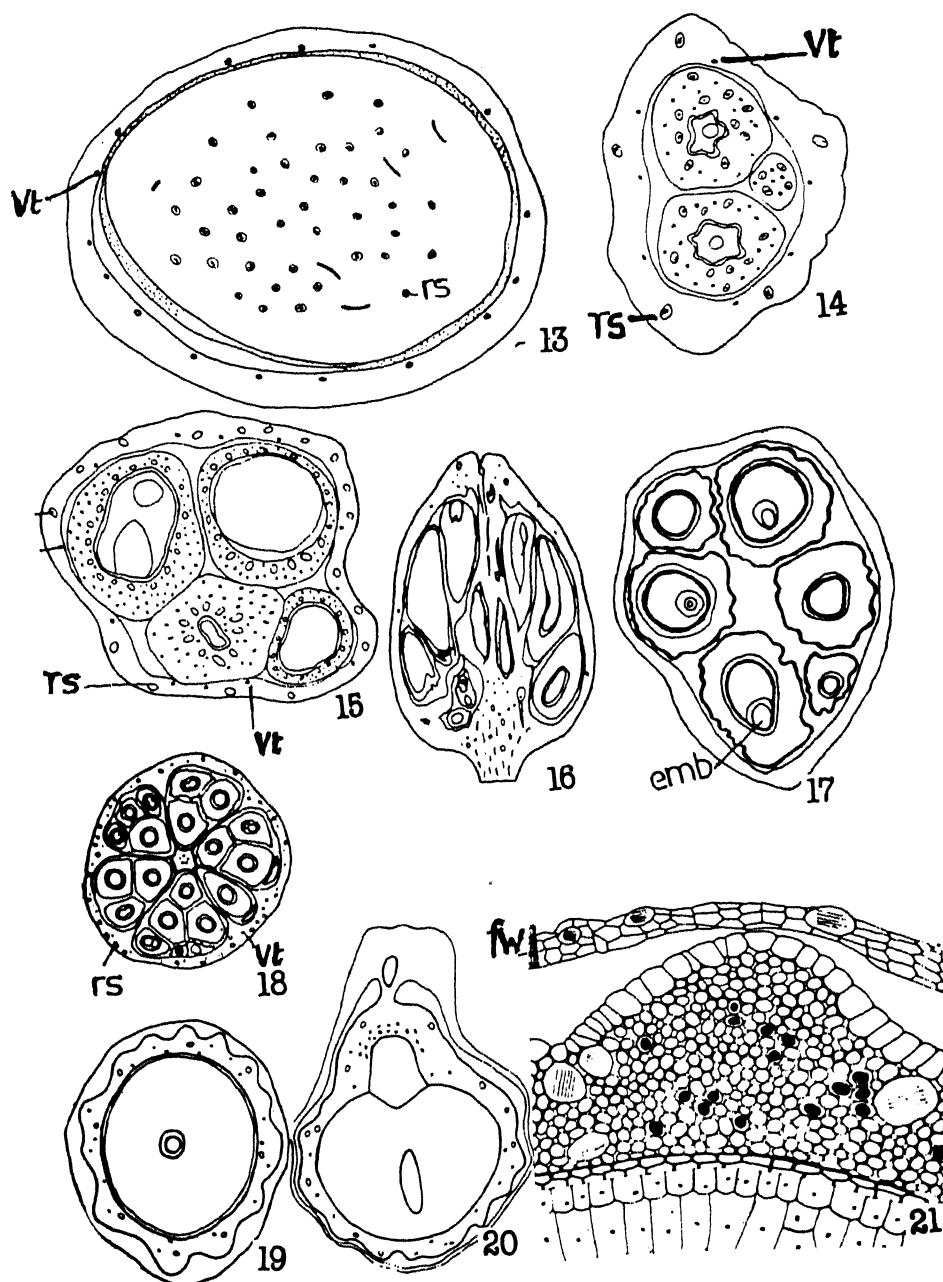
Figures 13–21. TS of fruit wall and seed. **13.** *Amorphophallus commutatus.* Fruit (×8). **14.** *Sauromatum pedatum.* Fruit (×8). **15.** *Arisaema tortuosum.* Fruit (×8). **16.** *Cryptocoryne spiralis.* Gross view of fruit in LS (×8). **17.** *Pistia stratiotes.* Fruit (×20). **18.** *Cryptocoryne spiralis.* Gross view of fruit in TS (×8). **19–21.** *Typhonium cuspidatum.* Fruit in gross view. **19.** TS (×8). **20.** LS (×8). **21.** Sector of fruit wall (×100). (rs, Raphide sac; Vt, vascular strands; emb, embryo; fw, fruit wall).

ends; cells tubular, pigmented with cutinised walls. Endosperm cells squarish to polygonal, thin walled (figure 11), densely filled with simple or compound, variously shaped, many times irregularly angled starch grains with prominent central hilum. Peripheral aleurone layer prominent.

Theriophonum indicum Schott

Fruit: Unilocular, 4–5 seeded; seeds globose or vaguely lobed.

Fruit wall: About 10 layered in radial extent (figure 7). Outer and inner epidermal cells rectangular, radially oriented. Ground tissue with a ring of vascular strands situated in the mid part of fruit wall. Raphide sacs circular, arranged in a ring in the outer part of fruit wall. Ground tissue cells hexagonal to rectangular, tangentially arranged, full of starch grains.

Seed: Testa more massive than fruit wall, slightly lobed at places; outer and inner epidermal cells rectangular; ground tissue cells full of starch grains; raphide sacs numerous, forming an almost continuous ring towards inner face; pigmented cells numerous, scattered singly and in groups. Tegmen degenerated, with two wavy layers of cuticle. Aleurone prominent, cells squarish to rectangular with prominent nuclei; inner endosperm cells radially enlarged, polygonal to hexagonal with abundant, simple or compound, circular starch grains with central round hilum.

Typhonium cuspidatum Decaisne

Fruit: Round one-seeded with papery pericarp, seed stalked, erect, stalk white (figure 20). Testa ribbed, star like in appearence in TS (figure 19); tegmen reduced to thick layer of cuticle. Embryo apical.

Fruit wall (figure 21):   Four to five layered in width; epidermal cells rectangular, longitudinally extended with cutinised outer tangential walls; ground tissue 2–3 layered, with a ring of enlarged raphide sacs, cells hexagonal, containing starch grains, vascular strands inconspicuous.

Seed (figure 21):   Outer epidermal cells of testa radially enlarged rectangular, appear empty; inner epidermal cells rectangular, tangentially extended, narrow; ground tissue cells with starch grains; raphide sacs enlarged; pigmented cells common, mostly distributed singly. Tegmen mostly reduced to a thick layer of cuticle, at places two layered, with rectangular, highly radially compressed cells. Aleurone cells squarish, with less starch grains; inner endosperm cells enlarged, oval to hexagonal, full of starch grains.

Subtribe: Arisaematinae

Arisaema tortuosum Schott

Fruit: Fruitlets densely aggregated on the fleshy globose to elongated inflorescence axis, elongated, 4–5-gonous, one chambered, 4–5 seeded (figure 15) with flat top having stigmatic residue in centre. Seed elongated, globose to angular attached basally through large white funicle.

Fruit wall (figure 8):   Outer and inner epidermal cells rectangular, tangentially extended in cross sectional view; outer epidermal cells polygonal, inner hexagonal

and longitudinally oriented in surface view, walls cutinised, cuticle slightly thicker on outer tangential walls of outer as well as inner epidermis. Ground tissue 4–6 layered, cells large, polygonal to hexagonal, filled with starch grains. Raphide sacs common, ovoid; vascular strands small, circular, in a ring situated in the inner half of fruit wall (figure 15).

Seed (figure 8): Testa and tegmen of variable thickness at different levels of seed, generally massive towards base and narrowed towards the top (figure 15). Outer epidermal cells of testa rectangular, tangentially oriented those of inner very much narrowed (figure 8). Ground tissue about 10–12 layered in width in the middle part of seed; cells large, polygonal, densely filled with starch grains; large solitary cells filled with pigmented material seen in mid ground tissue (figure 8); a ring of large, circular raphide sacs situated toward inner side of ground tissue (figure 15). Tegmen 2–3 layered in mid part of seeds, wider at base, cells tubular, slightly sclerotic (figure 8). Endosperm cells polygonal, thin walled, densely filled with simple to compound, variously shaped starch grains with central prominent hilum.

Subtribe: Cryptocoryninae

Cryptocoryne spiralis Fisch

Fruit: Fleshy, round, composite, hexalocular syncarpium with about 6 basally attached erect seeds per locule (figures 16, 18).

Fruit wall: About 12 layers in width (figure 3); outer and inner epidermal cells narrow, rectangular, tangentially oriented, outer cells with cutinised outer and inner tangential walls. Hypodermis not sharply defined, single layered, cells rectangular tangentially oriented. Vascular strands in a single ring, situated in middle of fruit wall, ground parenchyma cells oval to rectangular, tangentially oriented. Raphide sacs common, large, oval, subhypodermal ring prominent (figure 18). Pigmented cells occasional. Fruit wall notched at septal areas; septae thin, cells tubular radially oriented. Central column pentangular with a group of 10 vascular strands.

Seed: Roughly tetra- to pentangular in TS (figure 18), bitegmic; testa 2–3 layered, surface uneven, containing a ring of prominent protruding raphide sacs (figure 3); tegmen two layered, cells tubular, tangentially oriented, those of inner layer tannin filled. Endosperm completely filling the seed cavity, cells circular to angular, thin walled, densely filled with starch. Embryo well developed with raphide sacs and starch grains in the cotyledon.

C. cognata

Fruit and seed structure almost similar to that of *C. spiralis*. It differs from *C. spiralis* in having 2–3 rows of raphide sacs in fruit wall and about 10 layered testa and completely reduced tegmen.

Lagenandra ovata (L) Thw

Fruit: Fruitlets, irregularly lobed, unilocular, arranged densely on the fruiting axis; seeds 1 to few, attached basally.

Fruit wall: About 10 layered in width (figure 6). Epidermal cells, cubical. Hypodermis not distinct. Ground tissue cells loosely arranged, raphide sacs few,

situated in the middle of fruit wall. Tannin cells occasional. Vascular strands inconspicuous.

Seed (figure 6): Testa 3–6 layered in width, lobulate, each lobule internally supported by a raphide sac; pigmented cells pretty common. Tegmen 3 layered, cells thick walled, tubular, tangentially oriented, innermost layer tannin filled. Endosperm cells thin walled, densely filled with starch grains.

Subfamily: Pistioideae

Pistia stratiotes Linn

Fruit: Unilocular, 4–5 seeded (figures 4, 17). Seeds oblong with shallow longitudinal ribs.

   Fruit wall (figure 5) 6–8 layered in width. Epidermal cells rectangular. Ground tissue of rectangular to hexagonal, starch filled cells with a single ring of small vascular strands situated in the middle, 1–2 rings of distantly placed druses and occasional raphide sacs.

Seed (figure 5):   Testa massive, externally irregularly lobed. Epidermal cells filled with pigmented material, outer papillate, inner rectangular, cutinised; ground tissue massive, about 15 layered, cells oval to circular, loosely arranged having slightly thick walls; druses circular in 1–2 distantly placed rings, often associated below furrows of outer epidermis (figure 5); vascular strands and raphide sacs not conspicuous. Tegmen 1–2 layered, cells highly cutinised, rectangular, filled with pigmented material, often degenerating to a thick layer of cuticle. Endosperm of polygonal cells, filled with circular starch grains. Embryo apical, cotyledonary cells filled with starch grains.

4.   Discussion

The fruits in Araceae are berries. Amongst the species studied, they are composite in *Cryptocoryne* and simple in others. The thin pericarp provides few charactors of diagnostic value. Amongst these, the presence/absence and the arrangement of vascular strands, and the arrangement of raphide sacs/druses could be mentioned. On the other hand, most of the diagnostic features appear to be centered in the seed. Its smooth versus lobed/ribbed nature, the extent of development of testa and tegmen, the manner of distribution of raphide sacs, druses and pigmented cells in the testa offer significant features of taxonomic importance.

   The populations of *Amorphophallus commutatus* and *Aglaonema commutatum* studied here are habitual apomicts. In them, the fertilization fails and the enlarging embryo sac cavity gets filled with chalazal nucellar proliferation which produces along its periphery two or more shoot buds with associated adventitious roots as the fruits mature. The proliferating nucellus partially or completely digests the seedcoats and gets fully laiden with starch. It contains abundant raphide sacs which are entirely lacking from the endosperm of other investigated species.

   It would not be justifiable to discuss the bearing of fruit and seed anatomy on the systematics of Araceae based on this restricted work. However a few note worthy features could be indicated.

   Subfamily Aroideae, tribe Areae is well represented by *Theriophonum indicum*,

Typhonium cuspidatum and *Sauromatum pedatum* belonging to subtribe Arinae; *Arisaema tortuosum* belonging to subtribe Arisaematinae and *Cryptocoryne spiralis* and *Lagenandra ovata* belonging to subtribe Cryptocaryninae. All have closely arranged 1 to many seeded, regularly to irregularly angled or lobed berries which are laterally connated to form syncarpium in *Cryptocoryne*. The tegmen disintegrates in members of subtribe Arinae but is persistant in representatives of Arisaematinae and Cryptocoryninae. The testa is massive in members of subtribe Arinae and Arisaematinae but is thin in members of Cryptocoryninae. It is star shaped in *Theriophonum* and *Typhonium* and is ribbed due to the protrusion of raphide sacs in members of Cryptocoryninae. It is smooth in *Sauromatum* of Arinae and *Arisaema* of Arisaematinae.

This diversification in fruit and seed anatomical characters obviously supports Engler's (1920a) treatment of dividing tribe Areae into number of subtribes as against Hatchinson's (1959) treatment where all the subtribes are directly treated under his tribe Areae.

Subfamily Pistioideae representing monotypic *Pistia stratiotes* is distinguished from other subfamilies by abundance of druses in the fruit wall and the seed coat which are lacking in studied members of other subfamilies where their place is taken over by profusion of raphide sacs. These along with other differential features justify the separation of this species into a distinct subfamily or a tribe as per suggestion of Engler (1920b) and Hutchinson (1959) respectively.

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Cyperaceae Indiae Australis Precursores — A novelty in *Eleocharis* R.Br. and its vegetative anatomy

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Abstract. One new species of *Eleocharis* is described and illustrated. Its anatomical characters though agree with those of the genus it is observed to differ by certain number of distinct features.

Keywords. *Eleocharis*; vegetative anatomy.

Eleocharis andamanensis *Govind.* **sp. nov.** — *Series Mutatae Svens.* (figure 1)

Eleocharis acutangula (Roxb.) Schult. affinis sed ab ea differt tuberibus late ovatis quae tecta sunt late ovatis et multinervis hyalinis squamis, culmis teretibus, glumis stramineis anguste hyalinis et marginatis setis parce scabribus flavis bis nucibus longioribus, basi stylis nucibus continuo, cellulis epicarpii verticulatim oblongis et hexagonis quae in indistinctis seriebus apparent, base styli quadrate aliquantum tessellata et in distincta cellulae serie distributa, nucibus flavis cum persistente stylo, base styli triquetre et nucibus continuo.

Perennial herbs, stoloniferous; stolon ending in beaked asymmetrical broadly ovate tubers; tubers covered with membranous hyaline broadly ovate multinerved scales, 8–9 × 5–6 mm. Culms erect, tufted, rather slender, terete throughout, spongy, irregularly nodulose, 15–30 (−35) cm × 0·5–1 mm. Sheaths membranous, deliquescent, Spikelets narrowly elliptic ovate, terete, broader than culms, acute, many flowered, dusky green, 1·5–2·5 cm × 1·5–2 mm. Glumes firm, stramineous, 4 ranked, adpressed, not convolute when dry, oblong, rounded at apex, faintly medianly 1 nerved with many nerves in each half, non keeled; margins hyaline without brown zonation, 3·5 4 × 1·5 mm. Bristles 6–7, yellow, rather unequal, 2 times longer than nut, brown, sparsely retrorsely scabrous. Stamens 2–3; anther linear ovate, *circa* 2 mm long with ovate acute apical prolongation. Nut turgidly biconvex, ribbed or ribless at margin, elliptic ovate, slightly narrowed into neck not forming annulus, yellowish brown with persistent style, 1·5–1·6 × 1 mm; epicarpic cells minute arranged in several vertical rows on each face; cells longitudinally oblong-hexagonal, indistinct thus appearing smooth; stylopodium persistent, continuous with nuts, usually triquetrous, deltoid, very short, nearly half as wide as nut with quadrate somewhat tessellated rather distinct outer cells.

Govindarajalu 11725, on the way to Mount Harriot, Andamans (type CAL); Isotypes: 11725 A–E; 11725 A (CAL); 11725 B (BSI); 11725 C (DD); 11725 D (BLAT); 11725 E (MH); Paratype: Govindarajalu 11756, on the way to Wandoor, Andamans (CAL).

Related to *E. acutangula* (Roxb.) Schult. but differs by the presence of broadly ovate tubers covered with broadly ovate multinerved hyaline scales, terete culms, stramineous narrowly hyaline margined glumes, sparsely scabrid yellow bristles 2
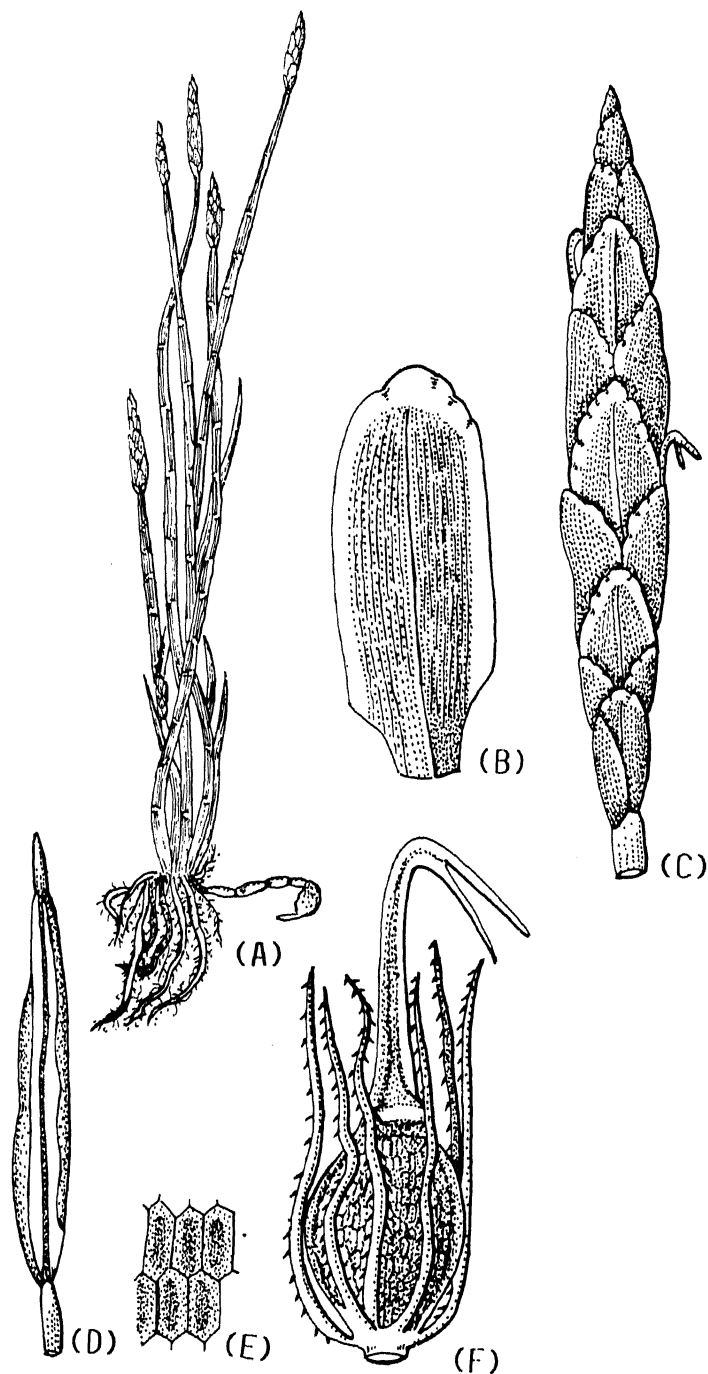
Figure 1. *Eleocharis andamanensis* Govind. *sp. nov.* **A.** Habit (×0·5). **B.** Glume (×15). **C.** Spikelet (×6). **D.** Anther (×42). **E.** Epicarpic cells, diagrammatic. **F.** Nut (×30). (from Govindarajalu 11725, type).

times longer than nuts, style base continuous with nuts, vertically oblong-hexagonal epicarpic cells occurring in many indistinct rows, quadrate somewhat tessellated distinct cell rows of style base, yellowish brown nuts with persistent style, triquetrous style base continuous with nuts.

Vegetative anatomy

For methods and other related information see Govindarajalu (1966, 1968a, b, 1969) and Metcalfe (1971). The descriptive terms proposed by Metcalfe and Gregory (1964) and the typological characterization of vascular bundles and metaphloem recognized by Cheadle and Uhl (1948a, b) are followed here.

Sheath

Abaxial surface (figure 2H): Epidermal cells as in *E. spiralis* (Govindarajalu 1975). Stomata (length 37·7–40·5 μm; width 17·5–19·6 μm); interstomatal cells sinuous. Silica cells occurring in a single discontinuous row each one of them possessing several more or less indiscriminately arranged small silica-bodies (figure 2I); large silica deposits of irregular size and shape rarely present in some of the intercostal cells (figure 2G) as in *Rhynchospora* (Govindarajalu 1969, 1975); nodular silica-bodies also occasionally present (figure 2F).

TS sheath (figure 2D, E): Epidermal cells as in *E. dulcis* (Govindarajalu 1975). Cuticle thin, *circa* 1·8 μm in thickness. Sclerenchyma strands (height 13·5–18 μm; width 13·5 μm) as in *E. acutangula* (Govindarajalu 1975). Air-cavities absent. Vascular bundles *circa* 9, large (type III A) with protoxylem lacunae and small (type I) possessing crescent shaped sclerenchyma at metaphloem pole; metaxylem vessel elements (*circa* 18 μm in diameter); metaphloem as in *E. atropurpurea* (Govindarajalu 1975). Bundle sheaths single layered, complete in all vascular bundles; parenchymatous in small vascular bundles; fibrous in large vascular bundles. Secretory cells common.

Culm

Epidermis, surface view: Cells usually with oblique end walls, other details see *E. acutangula* (Govindarajalu 1975). Stomata, see sheath. Silica cells narrow, elongated, thin-walled, somewhat sinuous present in a single discontinuous row and each cell containing 3–4 cone shaped silica-bodies surrounded by satellites.

TS culm (figure 2A, C): Diameter of specimens examined *circa* 0·9 mm. Outline circular. Cuticle thin, *circa* 5·8 μm in thickness. Epidermal cells rather large, moderately thick-walled. Assimilatory tissue 1–2 layered and air-cavities 18–20 (other details as in *E. geniculata*; Govindarajalu 1975). Sclerenchyma strands (height and width 13·5–18 μm) (see *E. atropurpurea* and *E. spiralis*; Govindarajalu 1975). Vascular bundles *circa* 10 in number; 6 large (type III B) forming an inner ring, the remaining 4 small (type I) forming an outer ring; sclerenchyma present at metaphloem pole in large vascular bundles; metaxylem elements (18 μm in diameter), rounded in outline; metaphloem of 'regular type'. Bundle sheaths of large vascular bundles as in *E. dulcis* (Govindarajalu 1975). Secretory cells radially elongated, very common in hypodermis.
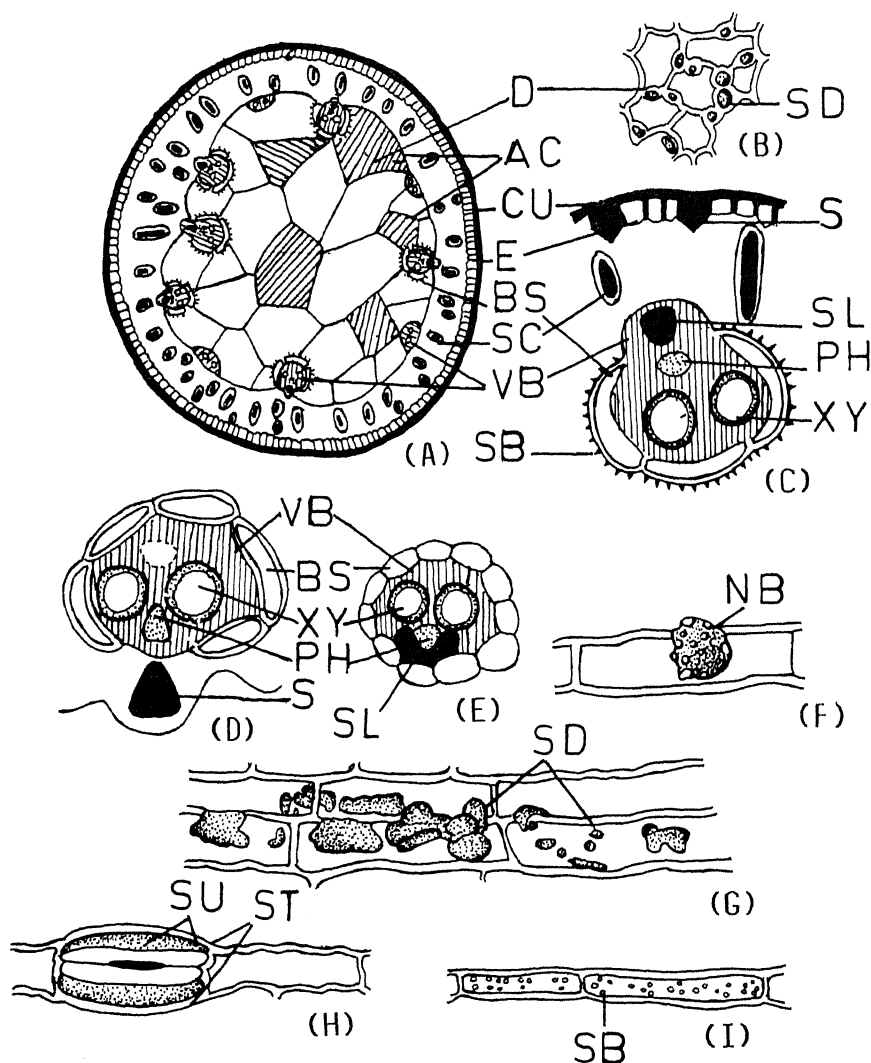
Figure 2. *Eleocharis andamanensis* Govind. **A.** Transection of culm, diagrammatic. **B.** Diaphragm cells of culm. **C.** Transection of culm large vascular bundle. **D.** Transection of large vascular bundle of sheath. **E.** Transection of small vascular bundle of sheath. **F.** Nodular silica-body of sheath. **G.** Silica deposit in intercostal cells of sheath. **H.** Stoma of sheath. **I.** Silica cells of sheath (**B–I** × 340) [from Govindarajalu 11725 E, (isotype) and 11756 (paratype)].

(AC, Air-cavity; BS, bundle sheath; Cu, cuticle; D, diaphragm and diaphragm cells; E, epidermis; NB, nodular silica-body; PH, metaphloem; PL, protoxylem lacuna; S, sclerenchyma strand; SB, silica-body; SC, secretory cell; SD, silica deposit; SL, sclerenchyma; ST, stoma; SU, subsidiary cell; VB, vascular bundle; XY, metaxylem element).

TS root: Diameter of specimens examined 0·3–0·4 mm. Exodermis: cells large, isodiametric with suberized outer tangential walls. Cortex very narrow consisting of 3–4 layers of compactly arranged large collenchymatous cells; cell walls gelatinous, excessively thickened. Starch grains present in cortex. Endodermis as in *E. acutangula*

(Govindarajalu 1975). Pericycle not distinct. Metaxylem and metaphloem as in *E. congesta* (Govindarajalu 1975); metaxylem elements (*circa* 29 μm in diameter)

Materials examined: Govindarajalu 11725 E (isotype) and 11756 (paratype).

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Cyperaceae Indiae Australis Precursores — Nova species in *Fimbristyle* (L.) Vahl and their vegetative anatomy

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Abstract. Two new species collected from South India are described and illustrated. Though the anatomical characters are in general agreement with those of the genus as a whole those that are specific are described.

Keywords. *Fimbristylis*; vegetative anatomy.

24. Fimbristylis pseudomicrocarya *Govind.* spec. nov. (figure 1)

Fimbristyle microcarya F.v.M. affinis, sed ab ea differt culmis brevioribus quadrangulatis vel quinque angulatis, foliis brevirobus et angustioribus fere laevigatus per marginem cum paucioribus nerviis, vaginis infimis foliis carentibus duo vel tres, inflorescentia simplice et breviore cum paucioribus apiculis, bracteis cum paucioribus nerviis et laevigato margine, spiculis linearibus oblongis (subacutatis) longioribus angustioribus cum multis floribus plerumque binatis, glumis chartaceis et semidistichis.

Annuals. Culms caespitose, filiform, 4–5 gonus, few-many, glabrous, rigid, erect, ribbed, sulcate, leafy at base, smooth throughout, (4–) 5–6 (−7) cm × 0·4–0·5 mm. Leaves few-many, filiform, glabrous, flat, acute, 3–7 nerved, almost smooth throughout margin, ligulate with thickened margin, stiff, usually erect, shorter than culms, (2−) 3–4 cm × 0·3–0·5 (−0·8) mm; uppermost sheaths leaf bearing, obliquely erect, ciliate at mouth; 2–3 basal sheaths leafless, glabrous, Anthela simple, obliquely erect, contracted consisting of usually 3–9 (−15) spikelets, 6–10 cm long. Bracts somewhat leaf like, ovate-lanceolate, as long as or shorter than inflorescence, stiff, erect, 3–5 nerved, smooth margined, non asperous, 4–10 mm long. Spikelets linear oblong, obtuse (subacute), usually paired or in threes, cinnamomeous brown, angular, many flowered, erect, very small, sessile, 3–4 × 0·8–1 mm. Glumes deltoid ovate, subacute, chartaceous, distichous in lower half and spiral in upper half with distinct scarious margin, glabrous throughout, shining, cymbiform, nerveless in each half, somewhat inflated by nuts, not spreading, mucronate, 1·2–1·3 (incl. mucro) × 1 mm; mucro erect or recurved, 0·1–0·2 mm long; keel 3 nerved (seemingly 1 nerved); nerves excurrent into mucro. Rhachilla winged, excavated. Stamen 1; anther linear oblong, obtuse at both ends, minute, spurred at base, 0·2–0·3 mm long. Style triquetrous, glabrous with slightly dilated pyramidal base, 0·4–0·5 mm long; stigma 3, glabrous, 0·2–0·3 mm long. Nut obovate, white, umbonulate, minutely stipitate, usually crystalline, trigonous, tricostulate with convex sides, smooth, 0·5–0·6 × (−0·3)0·4 mm; epicarpic cells in upper half distinct, transversely elongated-hexagonal occurring in 4–5 regular rows in each face appearing transversely lineolate.
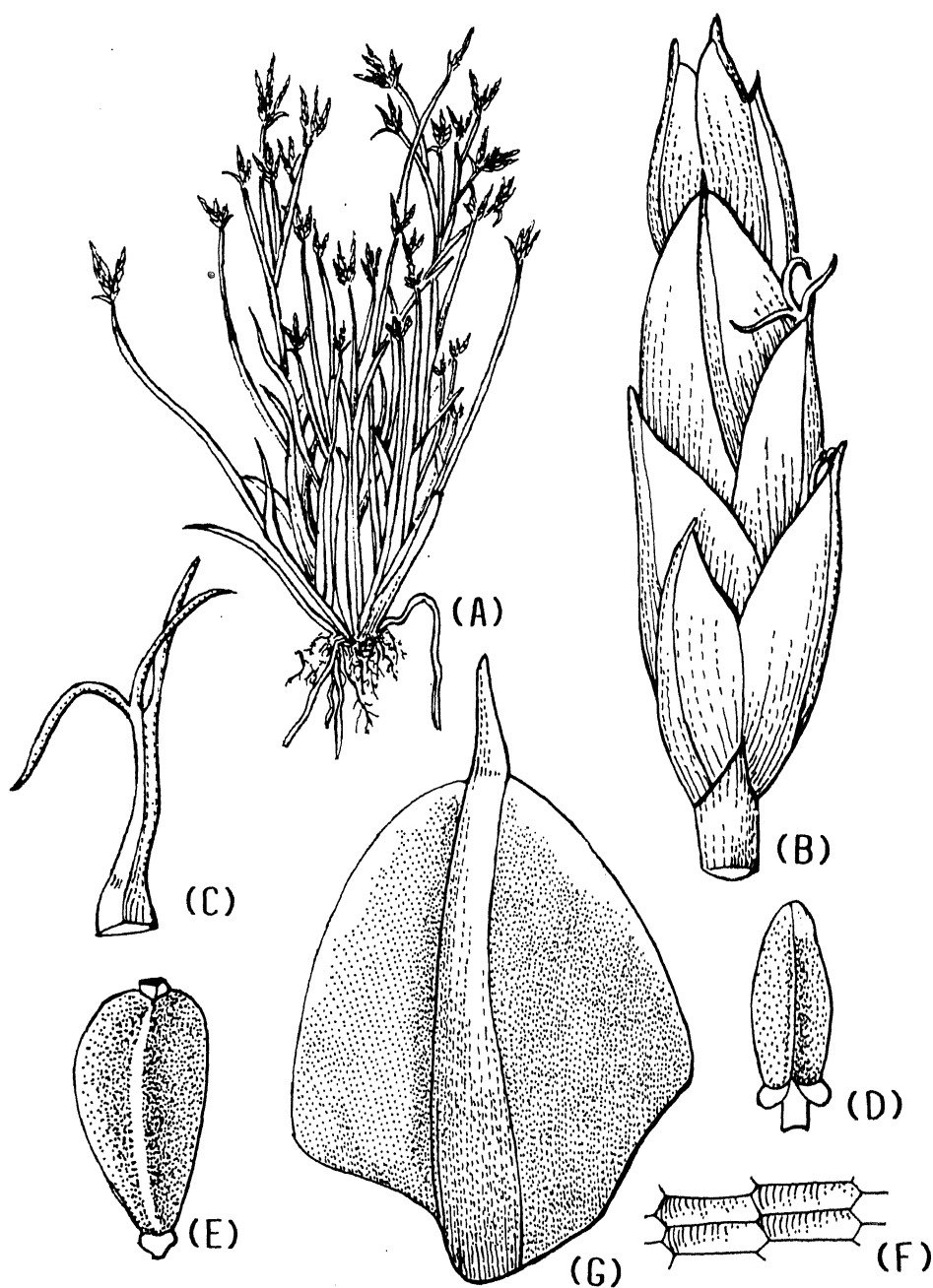
Figure 1. *Fimbristylis pseudomicrocarya* Govind.   **A.** Habit (× 0·5).   **B.** Spikelet (× 20).
C. Style and stigma (× 50).   **D.** Anther (× 65).   **E.** Nut (× 50).   **F.** Epicarpic cells,
diagrammatic.   **G.** Glume (× 54) (from Govindarajalu 13141, type).

Govindarajalu 13141, Bachapu, Someshwar, south Canara, Mysore State, not
common occurring in grasslands (*Holotype*: CAL); isotype (MH); *paratype*: 13182,
Kudremukh, south Canara, Mysore State, not common (BSI); 14817 C, Cliff view

forest, Nelliampathy, Pothundy, Kerala State, very common in grasslands (CAL); 14816, *ibid.* (BLAT); 14816 B (DD).

Notes: (i) This novelty very closely resembles *F. microcarya* F.v.M. to such an extent that it can easily be mistaken for the latter and therefore it is named as 'pseudomicrocarya'. (ii) This species occurs in grasslands of south Canara, Mysore State and Kerala State.

25. Fimbristylis hirsutifolia *Govind.*, spec. nov. (figure 2)

Fimbristyle bisumbellata (Forsk.) Bub. affinis sed angustioribus cum multum brevioribus basalibus foliis, bracteis multum brevioribus inflorescentia, parum maioribus glumis cum longiore arista, parum maioribus nucibus cum 15–20 epicarpicorum cellulorum seriebus ad quemque superficiem patenter dilute verticalibus striatis differt.

Annuals. Culms fascicled, stiff, erect, tetragonous, 4 ribbed and sulcate, smooth, glabrous, leafy at base, 8–12 cm × 0·3 mm. Leaves hirsutely hairy abaxially and at margin, acute, stiff, flat, much shorter than culms, ligulate, 6–9 nerved, 3–5 cm × 0·4–0·8 mm; sheaths hirsutely hairy, all laminiferous with brown membranous margin, obliquely truncate. Inflorescence compound, umbelliform, lax, patent consisting of 5–10 spikelets, 2–2·5 (–3) broad. Bracts much shorter than inflorescence, filiform, 1–1·5 cm long. Primary rays 4–8, glabrous, stiff, smooth, 1·5–2·5 cm long. Spikelets ovate lanceolate, solitary, acute, angular, erect, 4·5–5 × 1·5–2 mm. Basal glumes 1–2 empty. Glumes elliptic oblong or oblong ovate, obtuse, cinnamomeous brown, erect-obliquely erect, esquarrose, membranous and sides with many tannin striations resembling nerves, 2 (incl. arista) × 0·8–1 mm; arista 0·3–0·4 mm long; keel strongly 3 nerved; nerves excurrent into arista. Rhachilla shortly winged. Stamen 1; anther linear lanceolate (oblong), apiculate, minutely spurred at base, 0·6–0·7 mm long. Style flat, hairy throughout or papillose in lower half, dilated at base, 0·8–1 mm long; stigma 2, sparsely hairy in lower half, shorter than style, papillose, up to 0·6 mm long. Nut obovate-pyriform, yellow-brown, biconvex-planoconvex, opaque, minutely stipitate with or without thickened margin, umbonulate, 0·7–0·8 (incl. stipe) × 0·6–0·7 mm; epicarpic cells in upper half indistinct, shortly hexagonal-quadrate, occurring in 15–20 regular rows on each face appearing faintly vertically striated.

Govindarajalu 15480 A, Calicut University Campus (Botanic Gardens), not common occurring in semiwet habitats (Type: CAL); isotype (MH).

Note: Named on the basis of hirsute hairs present on the leaves.

Vegetative anatomy

For methods, descriptive terms, typological characterization of vascular bundles, classification of metaphloem, see Govindarajalu (1966, 1968a, b; 1975), Metcalfe (1971), Metcalfe and Gregory (1964) and Cheadle and Uhl (1948a, b).

Fimbristylis pseudomicrocarya Govind.

Lamina—Abaxial surface: Intercostal cells axially elongated, similar in size and
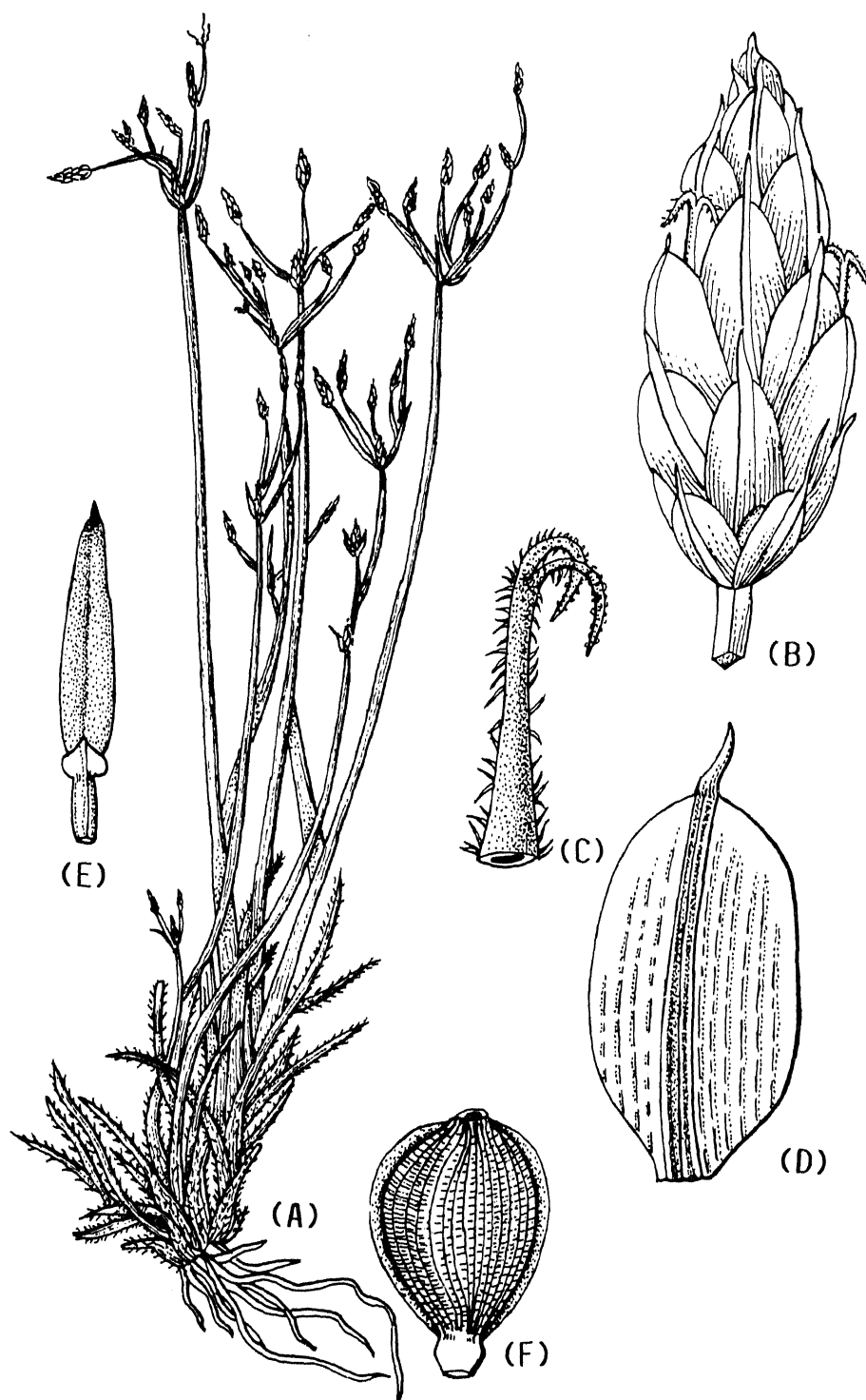
Figure 2. *Fimbristylis hirsutifolia* Govind. **A.** Habit (× *circa* 0·5). **B.** Spikelet (× 10). **C.** Style and stigma (× 43). **D.** Glume (× 22). **E.** Anther (× 40). **F.** Nut (× 30) (from Govindarajalu 15480 A, type).

shape; cell walls moderately thick, sinuous. Stomata (length 41·5–45 μm; width 22·5–27 μm), elliptic or elliptic oblong, paracytic; subsidiary cells low dome-shaped or parallel sided; interstomatal cells axially elongated with concave ends. Silica cells not common occurring in 1 (−2) discontinuous rows each one of them containing 2–3 cone-shaped silica-bodies surrounded by satellites.

Adaxial surface: Cells rather broad, variable in size and shape; cell walls thin, Stomata (length 28–32·5 μm; width 22·5–27 μm), broadly elliptic; subsidiary cells low dome-shaped; interstomatal cells short with concave ends. Other details, see abaxial surface.

TS lamina: Width of lamina examined 0·6 mm. Outline shallowly crescentiform without prominent midrib (figure 3B); margin rounded with small peg-like protuberance on adaxial surface. Cuticle over adaxial surface thicker than that of abaxial surface. Epidermis: adaxial row of cells conspicuously larger than that of abaxial, the former consisting of large inflated translucent cells. Bulliform cells, see adaxial epidermis (figure 3B). Sclerenchyma strands: abaxial rectangular (height 10·8–12·6 μm; width 18 μm); midrib strand inversely triangular (height 18 μm; width 27 μm); adaxial marginal strand pulviniform (height 18 μm; width 36 μm). Mesophyll, air-cavities and secretory cells, see Metcalfe (1971). Vascular bundles *circa* 12, large and small, all belonging to type I, not regularly alternating with each other; all arranged in a single row nearer to abaxial surface. Bundle sheaths 2 layered; outer sheath fibrous, inner sheath parenchymatous, both complete.

Culm—Epidermis, surface view: Cells axially elongated; cell walls moderately thick, sinuous. Stomata (length 45–49·5 μm; width 18–22·5 μm), narrowly elliptic oblong, paracytic; subsidiary cells parallel sided; interstomatal cells axially elongated with concave end walls. Silica cells over sclerenchyma strands not common occurring in a single discontinuous row and each one of them possessing 5–6 cone-shaped silica-bodies without satellites.

TS culm: Diameter along long axis of the material examined 0·4 mm. Outline subhemispherical (figure 3A). Cuticle thin. Epidermis: cells large, variable in size. Sclerenchyma strands (height and width 46·4–69·6 μm), pulviniform-subspherical. Vascular bundles of 2 different size with *circa* 15 large (type III A) and small (type I) bundles arranged more or less in one ring; few large vascular bundles penetrating into centre; metaxylem vessel elements (7·2–9 μm in diameter); metaphloem of 'intermediate type'. Air-cavities, assimilatory tissue, central ground tissue and secretory cells, see Metcalfe (1971).

Material examined: Govindarajalu 13141, Bachappu, Someshwar, south Canara, Mysore State (isotype): 14816 and 14817 C, Cliff view forest, Nelliampathy, Pothundy, Kerala State (paratypes).

Fimbristylis hirsutifolia Govind.

Lamina—Abaxial surface: Intercostal cells variable in size and shape. Stomata (length 40·5–45 μm; width 27–31·5 μm), broadly elliptic; subsidiary cells triangular or low dome-shaped. Silica cells broad, short, thin-walled occurring in 1–2 continuous rows, each containing usually 2 large cone-shaped silica-bodies almost
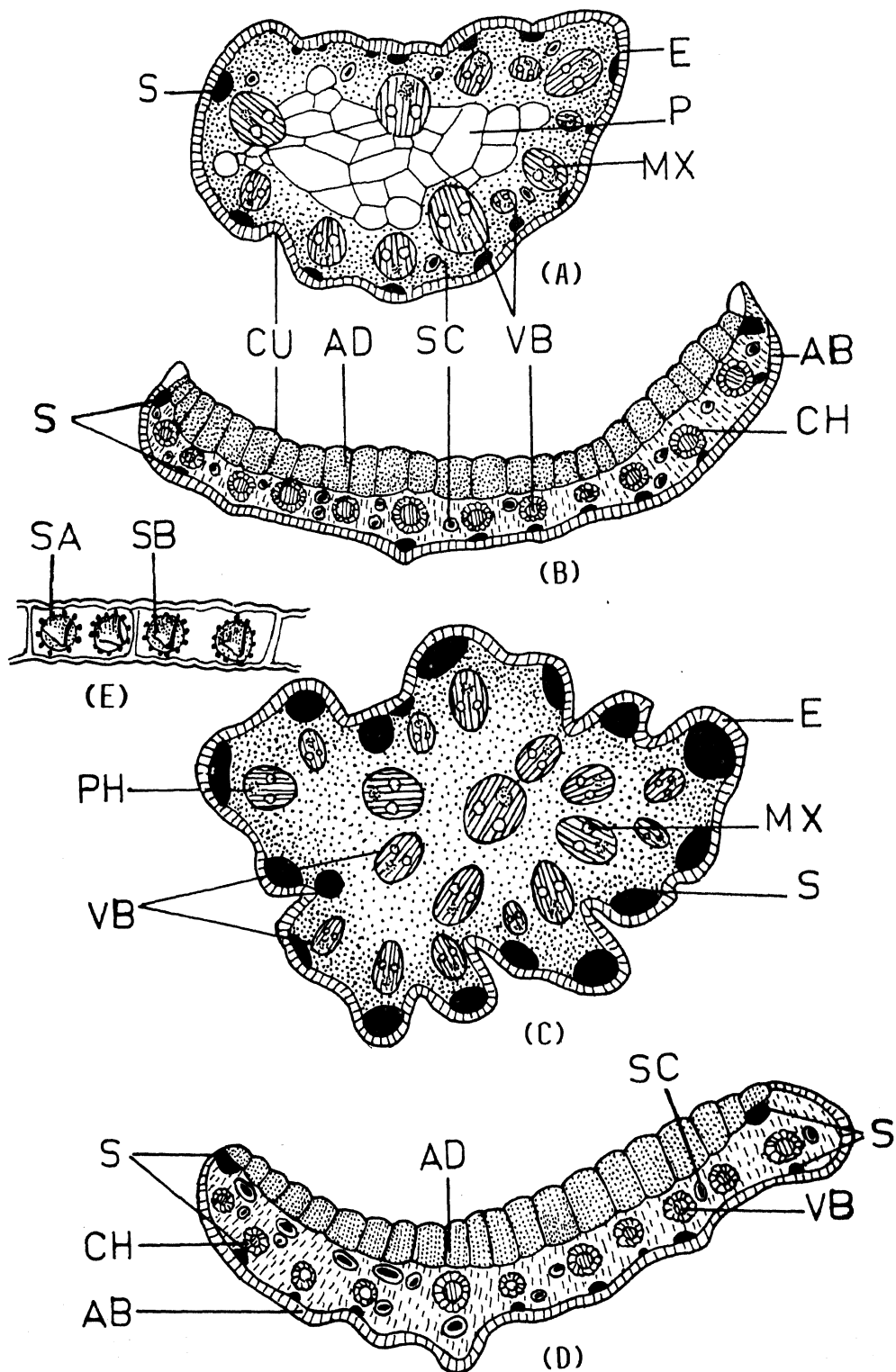
Figure 3.

filling the cell lumen and surrounded by satellites (figure 3E). Other details, see *F. pseudomicrocarya*.

Adaxial surface: Cells more or less similar in size and shape. Other details, see abaxial surface.

TS lamina: Width of lamina examined *circa* 1 mm. Outline shallowly crescentiform (figure 3D) without prominent midrib and with 7–8 very low abaxial ribs; margin rounded, one sloping downwards, the other upwards. Cuticle thick on either surface. Epidermis, see *F. pseudomicrocarya*. Scelerenchyma strands (height and width 12·6–14·4 μm), pulviniform. Bulliform cells, see adaxial surface (figure 3D). Vascular bundles *circa* 11 arranged in one row nearer to abaxial surface; other details, see *F. pseudomicrocarya*; metaxylem elements (13·5–18 μm in diameter). Mesophyll, air-cavities, metaphloem and secretory cells, see Metcalfe (1971).

Culm — Epidermis, surface view: Stomata (length 40·5–45 μm; width 36–40·5 μm), broadly elliptical; subsidiary cells low dome-shaped. Silica cells over strands not common occurring in a single more or less continuous row each one of them containing 3–4 cone-shaped silica-bodies; other details see *F. pseudomicrocarya*.

TS culm: Diameter of the culm examined *circa* 0·4 mm. Outline as in figure 3C, irregular with *circa* 12 prominent ribs and as many furrows. Epidermis: cells narrow, tangentially elongated. Sclerenchyma strands (height 22·5–31·5 μm; width 36–49·5 μm), other details, see *F. pseudomicrocarya*. Vascular bundles 17–18, variable in size forming two rings; 7 vascular bundles opposite to furrows forming an inner ring penetrating into centre, the remainder outer ring; all belonging to type III A without protoxylem lacunae; metaxylem vessel members (11·3–13·5 μm in diameter). Cuticle, metaphloem, central ground tissue and secretory cells, see *F. pseudomicrocarya*.

Material examined: Govindarajalu 15480 A, Calicut University Campus, Botanic Gardens (isotype).

Acknowledgements

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Figure 3. A and B. *Fimbristylis pseudomicrocarya.* **A.** Transection of culm ($\times$ 108). **B.** Transection of lamina ($\times$ 72). **C–E.** *F. hirsutifolia.* **C.** Transection of culm ($\times$ 108). **D.** Transection of lamina ($\times$ 88). **E.** Silica-cells, surface view ($\times$ 272) (from isotypes).
(AB, Abaxial epidermis; AD, adaxial epidermis; CH, radiating chlorenchyma; CU, cuticle; E, epidermis; MX, metaxylem; P, pith parenchyma; PH, metaphloem; S, sclerenchyma strand; SA, satellite; SB, silica-body; SC, secretory cell; VB, vascular bundle).

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A new species of *Jubula* Dumort. from Milam in Kumaon (western Himalaya)*

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Abstract. A new species of *Jubula* Dum., *Jubula himalayensis* sp. nov. has been described from the western Himalaya (on way to Milam glacier). The species is characterized by plants having monoecious sexuality, entire bracts and bracteoles, oval to spherical, homogeneous and refracting 4–7 oil bodies per cell.

Keywords. Bryophyta; Hepaticae; Jubulaceae; *Jubula himalayensis* sp. nov.

1. Introduction

According to the earlier reports, genus *Jubula* Dumort. of the subfamily Jubuloideae Klinggr. under the family Jubulaceae Klinggr. (Schuster 1979) was represented in India by *J. hutchinsiae* (Hook.) Dum. sub sp. *javanica* (St.) Verd. and *J. hattorii* Udar *et* Nath, the former being distributed in the eastern Himalaya and south India (Chopra 1938; Kamimura 1961; Hattori 1966, 1971; Udar and Nath 1978, 1979) and the latter being restricted to eastern Himalaya only. Plants of *Jubula* were collected from the locality of Bogdiyar on way to Milam glacier in district Pithoragarh (Kumaon Himalaya) and reported as associates of a moss (*Hookeria acutifolia* Hook. and Grev.) community by Tiwari *et al* (1987) but not described. Recently, during a collection trek to Milam glacier (Kumaon Himalaya) one of us (DS) came across those interesting plants of *Jubula* again, which on critical investigation showed considerable discrepancy in characters with the earlier known species of the genus and are clearly referable to a new species, *Jubula himalayensis* sp. nov., being described here.

The present communication also provides the extended range of distribution of *Jubula* from the eastern Himalaya and south India to the western Himalaya (on way to Milam glacier) at higher altitude (1850–2450 m).

2. Taxonomic description

Jubula himalayensis sp. nov. (figures 1–26)

Monoica magna, Caulis ad 6 cm longus, prostratus, pinnates ad bipinnates ramosus *Frullaniae* similis; Folia imbricata, apice acuminata, incurva unidentatis, 4–6 cellulae altus; cellulae superae et marginalis angustiora elongatus vel sub-rectangulata 15–33·75 × 7·5–18·75 μm et 18·75–26·25 (37·5) × (7·5) 11·25–16·85 μm respectus; trigonis parvis; Amphigastria magna, caule duplo ad quinquiens latiora,

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*Contribution New Series (Bryophyta) No. 237.
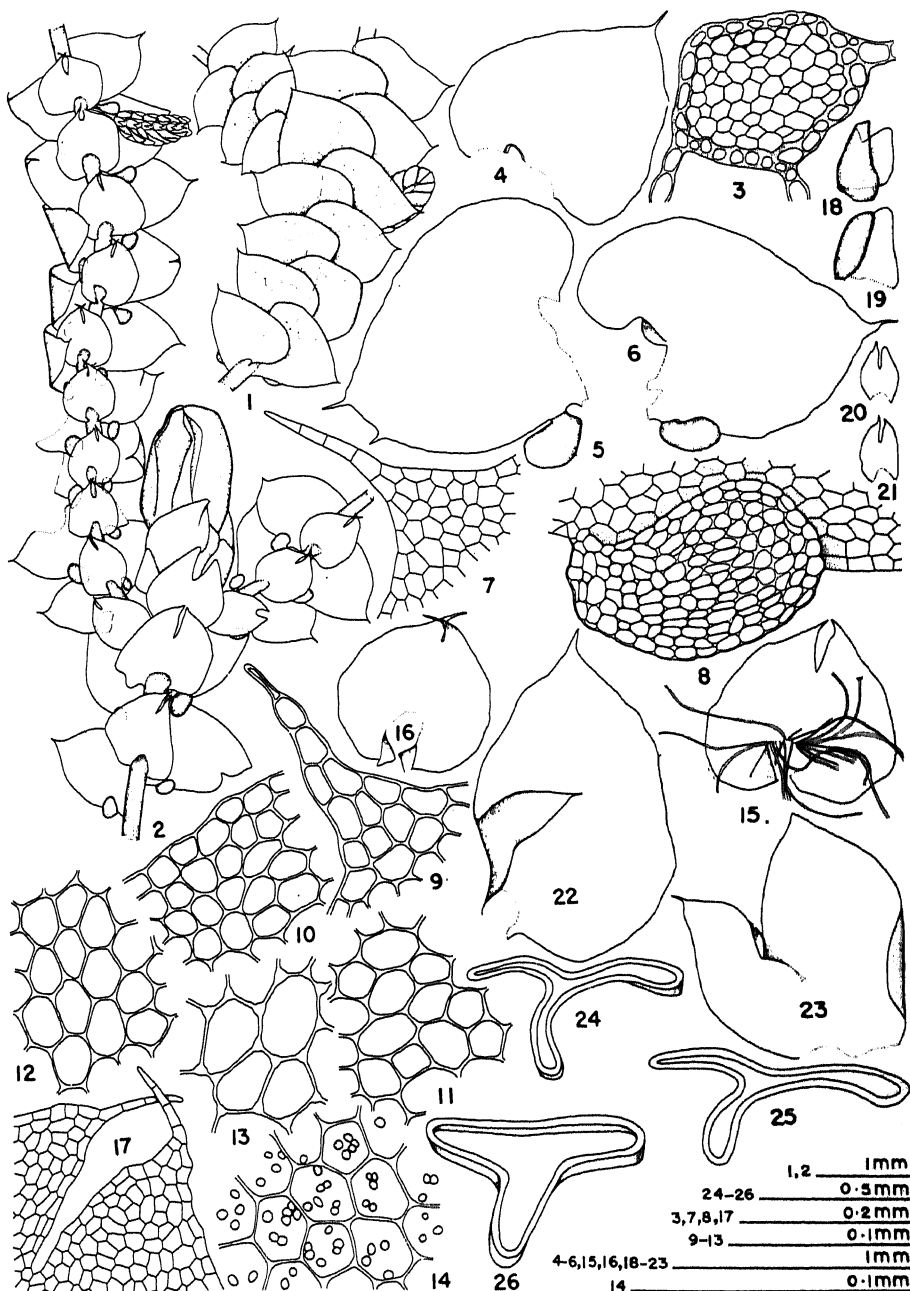
Figures 1–26. *Jubula himalayensis* sp. nov. **1.** A portion of plant in dorsal view. **2.** Portion of plant in ventral view with male and female branches. **3.** TS of stem with leaf and underleaf **4.** Leaf near the lateral branch emergence. **5 and 6.** Leaves. **7.** Apiculate Apex of leaf. **8.** Leaf lobule. **9.** Apical cells of leaf-lobe. **10.** Marginal cells of leaf-lobe. **11.** Middle cells of leaf-lobe. **12.** Antical basal cells of leaf lobe. **13.** Postical basal cells of the leaf lobe. **14.** Leaf lobe cells with oil-bodies. **15 and 16.** Under leaves. **17.** Underleaf apex (Cellular). **18 and 19.** Male bracts. **20 and 21.** Male bracteoles. **22.** Female bract. **23.** Female bracteole. **24–26.** Cross section of perianth apex, middle and base, respectively.

cordiformia ad suborbiculatus, apice 1/3–1/2 exciso biloba, lobis triangulatis apice longe setaceis, inter se cruciatim. Inflorescentia masculinus 6–12 bracteis jugis confertis, bilobis, lobis longe acuminatis; Inflorescentia feminea, terminalis in ramis lateralibus, bracteis et bracteolis disposita, bilobata, margine integro; Perianthium obovoideum glabrum 3 carinatum.

Plants medium to robust, 20–55 mm long and up to 2·23 mm wide, light to dark green, slender, prostrate, growing in mats on rock surface, branching terminal: of the '*Frullania* type', pinnate to bipinnate, branches replacing the leaf lobule. Stem 0·18–0·24 mm broad and 11–12 cells across diameter, differentiated into small (15–26·25 × 11·25–15 μm), quadrate to subquadrate thick walled, slightly pigmented cortical cells, and large (22·5–33·75 × 15–26·25 μm), angulate, rather thin-walled and non-pigmented medullary cells. Leaves incubous, horizontally spreading, insertion long and oblique, 1·14–1·20 mm long and 1·03–1·20 mm wide, ovate to sub-triangulate, apex incurved, cuspidate to acuminate, rarely obtuse with commonly 1(2) uniseriate, 4–6 cells high and 2–4 cells wide, incurved teeth, margin entire, antical margin strongly arched but seldom crossing the stem, the postical margin nearly straight or slightly arched; cells at apex 15–33·75 × 7·5–18·75 μm, narrowly elongate, at margin 18·75–26·25 (37·5) × (7·5) 11·25–16·85 (18·75) μm, narrowly rectangulate, at middle (18·75) 22·5–45 × (11·25) 18·75–26·25 μm, quadrate to subquadrate, thin-walled, at antical basal end 18·75–26·25 × 15–18·75 μm, subquadrate to isodiametric and at postical basal end 41·25–56·25 × 18·75–33·75 μm, large, subquadrate to broadly rectangulate with small trigones. Oil-bodies 4–7 per cell, oval (5 × 3·75 μm) to spherical (2·5–3·75 μm in diameter), smooth, homogeneous and refracting; leaf lobue saccate, somewhat distant and parallel to the stem, vertex rounded, mouth truncate. Underleaves contiguous, insertion on the stem strongly arching, longly decurrent, 0·53–0·7 mm long 0·7–0·92 mm wide, widely ovate, cordate to sub-orbiculate, somewhat wider than long and 2–5 times wider than stem, margin entire, apex 1/3–1/2 bilobed, apex of each lobe acute to acuminate and crossing each other, sinus narrow and 0·22–0·31 mm deep, cells at apex 18·75–30 × 5·6–11·25 μm, elongated; at margin 11·25–37·5 × 7·5–15 μm, subquadrate to narrowly rectangulate; at middle 18·75–33·75 × 11·25–18·75 μm, quadrate to rectangulate and at base 22·5–30 × 15–26·25 μm, rectangulate to ovate or sometimes isodiametric, trigones absent, Rhizoids arising from the base of the underleaf. Monoecious. Male inflorescence on lateral branches (not replacing the leaf lobule), of '*Radula* type', spicate; bracts in 6–12 pairs, ovate, up to 0·41 mm long and 0·41–0·45 mm wide; bracteoles ovate, small 0·28–0·31 mm long, 0·17–0·21 mm wide, 1/2 bilobed, lobes acute to acuminate, sinus narrow, base slightly decurrent, margin entire. Female inflorescence on the main axis or lateral branch ('*Radula* type') with two sub-floral innovations; female bracts in one pair, bract lobe obovate-oblong, 1·27–1·55 mm long and 0·96–1·33 mm wide, apex acuminate, margin entire; bract lobule small, up to 0·52 mm long and 0·21–0·24 mm wide, triangulate to oblong, acuminate, entire; bracteoles free, obovate to cordate, up to 1·17 mm long and 0·96 mm wide, 1/3–1/2 bilobed, sinus 0·52 mm deep and 0·59 mm wide, lobes elliptic to ovate, apex acute to acuminate, base cordate, margin entire. Perianth 1/2–3/4 emergent, obovate, dorsally compressed, 2·06–2·24 mm long and 1·03–1·30 mm wide, smooth, triplicate (2 lateral and 1 ventral), entire, apex rounded to sub-truncate with a short beak. Sporophyte not seen.

Type specimen deposited in LWU: 9853/88 (Holotype) *Jubula himalayensis* sp.

nov. Loc.: Bogdiyar to Lilam, on way to Milam glacier (western Himalaya), Alt.: 1850–2450 m, Lat.: ca. 30°N and Long.: ca. 80°E, Leg.: Deepak Sharma, Date: 18·6·88, Det.: S C Srivastava and Deepak Sharma. Habitat: on moist, shaded rock surface and crevices in a well sheltered ravine on the banks of a river; associated with *Marchantia* sp. *Metzgeria* sp. and *Plagiochila* sp.

2.1 *Specimens examined*

LWU 9847/88, 9849/88, 9850/88, 9852/88 and 9855/88. *J. himalayensis* sp. nov. Loc.: Bogdiyar to Lilam (western Himalaya) Alt.: 1850–2450 m, Lat.: ca. 30°N and Long.: ca. 80°E, Leg.: Deepak Sharma, Date: 18·6·1988, Det.: S C Srivastava and Deepak Sharma. Plants growing associated with *Jungermannia gollani, Marchantia* sp., *Metacalypogeia alternifolia, Metzgeria* sp., *Pellia* sp., *Plagiochila* sp. and *Riccardia* sp. Plants growing in mats on rocks and crevices in a well sheltered moist ravine on the banks of river Gori Ganga; LWU 000220/69, *Jubula hattorii* Udar *et* Nath (holotype), Loc.: Tiger hill, Darjeeling (eastern Himalaya) Alt. ca. 2590 m, Leg.: RU and Party, Date: 27·12·1969, Det.: RU and V Nath. Growing in moist shady places, epiphytic on bark of trees, associated with *Lepidozia reptans, Trichocolea, Thysananthus, Scapania, Lophocolea, Frullania* and mosses; LWU 7353/83, *Jubula*

Table 1.

Jubula hutchinsiae (Hook.) Dum. sub sp. *javanica* (St.) Verd. (Kamimura 1961)	*Jubula hattorii* Udar *et* Nath (Udar and Nath 1978)	*Jubula himalayensis* sp. nov.
Monoecious	Dioecious	Monoecious
Plant 30–50 mm long	Plants 15–40 mm long	Plants 20–55 mm long
Stem 7–8 cells across diameter	Stem 10–11 cells across diameter	Stem 9–11 cells across diameter
Leaves nearly as long as wide, 0·8–0·9 mm long and 0·65–0·8 mm wide. Apical cells 18–20 × 15–17 μm, basal cells 30–35 × 20–25 μm. Trigones small and indistinct	Leaves usually longer than wide, 0·8–1·4 mm long and 0·6–0·95 mm wide. Apical cells 8·14–16·28 × 8·14–12. 21μm, basal cells 20·35–36·63 × 16·28–28·49 μm. Trigones absent	Leaves as long as wide, 1·14–1·20 mm long and 1·03–1·20 mm wide. Apical cells 15–33·75 × 7·5–18·75 μm, basal cells 41·25–56·25 × (15) 18·75–33·75 μm. Trigones small and distinct
Oil-bodies 6–10 per leaf cell, ovoid, ellipsoidal or spindle-shaped, 7–9 × 4 μm with numerous minute globules	Oil-bodies 4–8 per leaf cell, spindle-shaped, 4·05–9·45 μm long and 4·05–3·4 μm broad, homogeneous with a refracting granule	Oil-bodies 4–7 per leaf cell, ovoid to ellipsoidal, 5 × 3·75 μm spherical, 2·5–3·75 μm in diameter, homogeneous and refracting
Underleaves always longer than wide, widely ovate 0·5–0·6 mm long 0·35–0·48 mm wide, 2 times wider than the stem, mostly with one tooth on both lateral margin	Underleaves always longer than wide (rarely vice versa), widely ovate 0·4–0·6 mm long 0·23–0·7 mm wide, 2–3 times wider than the stem with entire margin	Underleaves always wider than long (rarely vice versa), widely ovate to suborbiculate, 0·5–0·7 mm long 0·7–0·9 mm wide, 2–5 times wider than the stem with entire margin
Male bracts in 5–7 pairs, bracteoles developed up to the apex of the male inflorescence	Male bracts in 6–7 pairs, bracteoles not developed up to the apex of the male inflorescence	Male bracts in 6–12 pairs, bracteoles developed up to the apex of the male inflorescence
Female bracts and bracteoles with irregularly toothed margin	Female bracts and bracteoles with entire margin	Female bracts and bracteoles with entire margin

hutchinsiae (Hook.) Dum. sub sp. *javanica* (St.) Verd. Loc.: Perumalmalai (south India) Alt. ca. 2100 m, Leg.: RU and Party, Date: 29·9·1983, Det.: S C Srivastava and Deepak Sharma. Plants commonly occurring as calcicole, often epiphytic on *Dumortiera hirsuta*.

3.   Discussion

J. himalayensis approaches *J. hutchinsiae* sub sp. *javanica* in having monoecious sexuality but differs in the structure of female bracts and bracteoles which are entire margined in the former and dentate margined in the latter. *J. himalayensis* also simulates *J. hattorii* in having entire margined female bracts and bracteole but differs in sexuality, the latter being dioecious.

Further *J. himalayensis* is convincingly different from the two species in the structure of the oil bodies, which is oval to spherical, homogeneous and refracting in the former, spindle-shaped with a refracting granule in *J. hattorii* (Udar and Nath 1978, 1979), and ellipsoidal or spindle-shaped consisting of numerous granules in *J. hutchinsiae* sub. sp. *javanica* (Kamimura 1961).

A comparison of *J. himalayensis* with other two species has been shown in table 1.

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Cytology of hardwoods

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Abstract. Cytology of 51 woody species including 7 commercial timbers, belonging to Gamopetalae and Monochlamydeae have been carried out. Two genera (*Melodinus monogynous*, $n = 11$ and *Homonoia riparia*, $n = 22$) and 8 species (*Gardenia latifolia*, $n = 11$; *Ixora barbata*, $n = 11$; *Ligustrum sinense*, $2n = 46$; *Strophanthus wallichii*, $n = 11$; *Dolichandrone cynanchoides*, $n = 20$; *Beilschmiedia roxburghiana*, $n = 12$; *Persea gratissima*, $n = 12$ and *Phyllanthus discoides*, $n = 13$) have been counted for the first time. Variable chromosomes have been recorded for 7 species. In addition phenomena of cytological interest like structural hybridity due to reciprocal translocations (*Rauvolfia serpentina*, $2n = 22$ and *Broussonetia papyrifera*, $2n = 26$), B-chromosomes (*Breynia rhamnoides*, $n = 26 + 0 - 2B$ and *Joannesia princeps*, $n = 11 + 0 - 2B$) and cytomixis (*Serissa foetida*, $2n = 22$) have been detected. Abnormal microsporogenesis in a diploid tree of *Cleistanthus collinus* ($n = 10$) might be attributed to the disfunction of spindle apparatus.

Keywords. Chromosome number; hardwoods; cytomixis; structural hybridity.

1. Introduction

As a part of germplasm collection, a large number of exotic and Indian woody species of ornamental, medicinal and forestry importance have been introduced in the New Forest Division of Forest Research Institute, Dehradun. Keeping in view the fact that genepool analysis of plants forms a pre-requisite for undertaking future breeding programmes, the importance of cytological studies on such species hardly needs any emphasis. Although the attempts of Rao (1954, 1967), Nanda (1962), Haque (1984) and Singhal *et al* (1985) have yielded chromosome numbers of more than 200 species, but still a large number of species remain unworked. In addition, except for Singhal *et al* (1985), the earlier workers have only confined to the mere counting of the chromosomes and have not gone into the details of the meiotic behaviour. In view of the above facts the present work was undertaken on the members of Gamopetalae and Monochlamydeae, as a part of programme on chromosomal analysis of woody elements of the national flora.

2. Materials and methods

All the materials were collected from plants cultivated at New Forest and Botanic Gardens of Forest Research Institute, Dehradun. For meiotic studies, young floral buds were fixed in Carnoy's fluid and subsequently squashed in 1% aceto-carmine and made permanent in euparal. Pollen fertility was determined on the basis of their stainability with 1 : 1 glycero-acetocarmine and well filled nature.

3. Results

In all the investigated taxa belonging to 51 hardwood species, unless otherwise

mentioned, the course of meiosis as well as microsporogenesis are perfectly normal resulting into almost 100% pollen fertility. Only species showing cytological results of particular interest are dealt herewith.

3.1 *Serissa foetida* Lamk.

During meiosis in 76·1% of PMCs n = 11 has been recorded. Rest of the PMCs are involved in cytomixis, which is evident by the presence of cytoplasmic channels connecting 2–8 PMCs. Actual transfer of chromatin material is seen at prophase which results into PMCs with n = 9–12. Fall in pollen fertility (57·2%) is probably due to cytomixis.

3.2 *Ligustrum sinense* Lour.

The chromosome number 2n = 46 is confirmed. Based on x = 23 the species is diploid and shows irregular meiosis. In only 15% of the observed PMCs 23 bivalents are regularly constituted whereas in the rest, in addition to bivalents, other configurations are quadrivalents (1·85/PMC), trivalents (2·20/PMC) and univalents (1·40/PMC) and share 16·10, 14·34 and 3·04% of chromosomes, respectively. In all the analysed PMCs, the average frequency of bivalents per PMC is 15·30 and as many as 66·52% of chromosomes are involved in their formation. This leads to abnormal microsporogenesis in which besides normal tetrads, diads (9·1%), triads (7·4%) and polyads (11·6%) are observed. Pollen fertility is reduced to 62%.

3.3 *Rauvolfia serpentina* (Linn.) Benth. ex Kurz.

In a large number of plants 11 bivalents are observed at diakinesis and M–I. In case of one individual some multiple associations are observed in 26·31% PMCs at diakinesis and M–I, whereas in the rest, normal 11 bivalents are seen. Multiple associations include rings or chains of 4 and 6 chromosomes the average frequency per PMC for which is 0·18 (2 rings 0·02, ring 0·12, chain 0·04) and 0·09 (ring 0·05, chain 0·04) and share 2·4 and 3·5% of the chromosomes, respectively. As many as 94·1% of chromosomes of 57 PMCs form bivalents with 10·35 as an average frequency per PMC. Both the anaphases are normal. However pollen fertility is reduced to 72·5%. Interestingly, both normal and structural hybrid plants are morphologically indistinguishable.

3.4 *Breynia rhamnoides* Muell.-Arg.

Meiotic studies have revealed 26 bivalents at M–I. In 14·71% of PMCs 1–2 B-chromosomes are observed, the average frequency of these per PMC is 0·20. Pollen fertility is almost 100%.

3.5 *Cleistanthus collinus* Benth.

In a diploid tree at diakinesis and M–I, 10 bivalents are counted. Inspite of the

normal pairing laggards at anaphases and telophases are observed. Microsporo-genesis is also irregular due to the formation of dyads (19·8%), triads (9·5%) and polyads (10·2%) besides normal tetrads (60·5%). Pollen sterility is 30%.

3.6 *Joannesia princeps* Vell.

Eleven bivalents are regularly constituted at M–I. In 16·67% of PMCs 1–2 B-chromosomes are seen, the average per PMC of which is 0·28. Pollen fertility is 100%.

3.7 *Broussonetia papyrifera* Vent.

In a diploid tree (2n = 26) structural hybridity due to reciprocal translocations is evident by the presence of chains of 4 and 6 chromosomes in 21·8% of observed PMCs, the average frequency per PMC for which is 0·10 and 0·14 and share 1·5 and 3·3% of the chromosomes, respectively. In all, 95·2% of chromosomes in 49 analysed PMCs are involved in bivalents with 12·38 as an average frequency per PMC. In the rest of the PMCs, 13 bivalents are regularly constituted. Further distribution of chromosomes at A–I and A–II is normal. Pollen fertility is 82%.

4. Discussion

As a result of present investigations on 51 hardwood species, two genera* (*Melodinus monogynous*, n = 11 and *Homonoia riparia*, n = 22) and 8 species (*Gardenia latifolia*, n = 11; *Ixora barbata*, n = 11; *Ligustrum sinense*, 2n = 46; *Strophanthus wallichii*, n = 11; *Dolichandrone cynanchoides*, n = 20; *Beilschmiedia roxburghiana*, n = 12; *Persea gratissima*, n = 12 and *Phyllanthus discoides*, n = 13) have been investigated for the first time. Varied or additional chromosome counts have been recorded in 7 species. Information and comments about these species are given in table 1.

Present chromosome counts also include 7 commercial timbers (*Gardenia latifolia*, n = 11; *Alstonia scholaris*, n = 20; *Strychnos nux-vomica*, n = 22; *Stereospermum chelonoides*, n = 20; *Tectona hamiltoniana*, n = 18; *Cryptocarya amygdlina*, n = 12 and *Broussonetia papyrifera*, n = 13) of national importance. For 8 species viz., *Gardenia florida* (n = 11), *G. spathulifolia* (n = 11), *G. thunbergia* (n = 11), *Mussaenda frondosa* (n = 11), *Rauvolfia verticillata* (n = 11), and *Nicotiana glauca* (n = 12), *Aleurites fordii* (n = 11) and *Manihot tweediana* (n = 18) chromosomes have been counted for the first time from India. For 14 species (*Ligustrum lucidum*, n = 23; *L. nepalense*, n = 23; *Rauvolfia densiflora*, n = 22; *Buddleia lindleana*, n = 19; *Cordia cylindrostachya*, n = 18; *Brunfelsia americana*, n = 11; *Vitex peduncularis*, n = 17; *Alseodaphne kennani*, n = 12; *Persea bombycina*, n = 12; *Phoebe pallida*, n = 12; *Aleurites montana*, n = 11; *Gelonium multiflorum*, n = 11; *Macaranga denticulata*, n = 11 and *Sapium eugenifolium*, n = 22) the present counts are in confirmity with the earlier reports.

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*Based on Darlington and Wylie (1955); Index to plant chromosome numbers (1956 onwards), IOPB chromosome number reports (1965 onwards); Löve and Löve (1961, 1974, 1975); Fedorov (1969) and selected references from Biological Abstracts.

Table 1. Variable chromosome records.

Taxa	Previous record (2n)	Present record (2n)	Remarks
*Viburnum punctatum**	16	18	Based on the original base number (x = 9) of the genus
Ixora rosea	33	22	First report of deploid cytotype
Vernonia divergens	18	20	Diploid cytotype based on x = 10
*Tectona hamiltoniana**	24	36	Earlier report proves to be erroneous
Piper unguiculatum	28	26	Diploid cytotype based on x = 13
Cleistanthus collinus	22	20	Aneuploid at diploid level
Mallotus nepalensis	44	88	First record of Octaploid cytotype

*Species already investigated from New Forest.

The presence of B-chromosomes is detected in *Breynia rhamnoides* (n = 26 + 0 − 2B) and *Joannesia princeps* (n = 11 + 0 − 2B) which are tetraploid and diploid, respectively. In both of these species Bs are smaller than the A chromosomes. In PMCs with 2Bs, at M–I these often lie quite close to each other, showing a tendency to pair as in the case of *Festuca pratensis* (Bosemark 1950). This phenomenon may be attributed to stickiness or some homology between the B-chromosomes.

The occurance of structural hybridity in two diploid species viz., *Rauvolfia serpentina* (2n = 22) and *Broussonetia papyrifera* (2n = 26) is quite interesting. The formation of two multiple associations of 4 chromosomes (in 1·75% PMCs) coupled with the formation of ring or chain of 6 chromosomes (in 8·77% PMCs) in *R. serpentina* and the existance of chain of 6 chromosomes (in 14·3% PMCs) in *B. papyrifera* indicate that heterozygosity for interchanges is existing for atleast 4 and 3 pairs of chromosomes, respectively. In both the species, distribution of chromosomes at anaphases is normal, thus the existence of pollen sterility (28% in *R. serpentina* and 18% in *B. papyrifera*) in these might be due to the preponderance of successive type of saggregation of multiple associations and/or some other cryptic abnormalities (Bedi and Gill 1982).

From among the presently studied species intra specific polyploidy is seen in the Indian members of 8 species viz., *Ixora rosea* (2x, 3x), *Jasminum flexile* (2x, 4x), *Alstonia scholaris* (2x, 4x), *Plumeria alba* (2x, 3x), *Rauvolfia serpentina* (2x, 4x), *Strychnos nux-vomica* (2x, 4x), *Aleurites moluccana* (2x, 4x) and *Mallotus nepalensis* (4x, 8x) of these the presently recorded additional cytotypes are *I. rosea* (2n = 2x = 22), *A. moluccana* (2n = 4x = 44) and *M. nepalensis* (2n = 8x = 88).

The existance of multiple associations in *Ligustrum sinense* (2n = 46) as earlier suggested by Bedi and Bir (1985) might be due to reciprocal translocations or due to the fact that basic number x = 23 for the species in secondarily evolved from a lower number. Another diploid tree species *Cleistanthus collinus* (n = 10), which inspite of normal pairing at diakinesis and M–I, shows laggards at anaphases and telophases and also abnormal microsporogenesis. These abnormalities might be due to the malfunctioning of the spindle (cf. Stebbins 1971).

The phenomenon of cytomixis with the actual transfer of chromatin material and existence of hypo- and hyper ploid PMCs in *Serissa foetida*, which is a normal diploid plant might be under some genetic control as postulated by Brown and Bertke (1974). However the possibility of incomplete cytokinesis during premeiotic mitosis or defective cell wall formation etc. (Sarvella 1958) cannot be ruled out.

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Effect of L-methionine sulphoximine on the enzymes of nitrogen metabolism in barley leaves

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Abstract. L-methionine sulphoximine, a potent inhibitor of glutamine synthetase decreased nitrate reductase activity by 50% at the end of 12 h of treatment while nitrite reductase was insignificantly affected. By 3 h the inhibition of glutamine synthetase activity was complete. Elevated levels of ammonia induced by L-methionine sulphoximine did not influence glutamate dehydrogenase. It is inferred that ammonia accumulation does not affect photosynthetic electron transport which supplies reducing power to nitrite reductase. The failure of glutamate dehydrogenase activity to be induced by high ammonium levels shows that it is not involved in the process of ammonia assimilation in the leaves.

Keywords. Ammonia; methionine sulphoximine; nitrate reductase; nitrite reductase; glutamate dehydrogenase; barley.

1. Introduction

Glutamine synthetase (GS, EC 6·3·1·2) is the principal enzyme involved in the assimilation of ammonia arising from various sources in the plant system (Kumar and Abrol 1990). Inhibition of GS activity by L-methionine sulphoximine (MSO) in photorespiring tissues leads to increased ammonium levels (Martin *et al* 1983; Kumar *et al* 1984). Accumulation of toxic levels of NH_4^+ may result in uncoupling of photophosphorylation and consequently inhibition of CO_2 fixation (Platt and Anthon 1981; Achhireddy *et al* 1983). This view was later contradicted by Ikeda *et al* (1984) and Walker *et al* (1984) who showed that MSO-induced NH_4^+ accumulation did not affect CO_2 fixation.

Nitrate reductase (NR) and nitrite reductase (NiR) depend on the products of photosynthesis and photosynthetic electron transport, respectively, for the supply of reducing power (Beevers and Hageman 1983). There are no reports in the literature regarding the effects of MSO-induced ammonia accumulation on NR and NiR. Similarly, it is not known whether high amounts of ammonia accumulating as a consequence of MSO treatment, induce glutamate dehydrogenase (GDH) activity. The objective of the present study was to examine the effect of MSO on the activities of the enzymes of nitrogen assimilation in barley leaves.

2. Materials and methods

Barley (*Hordeum vulgare* L. cv DL-157) seedlings were grown in cement pots (38 × 72 cm) filled with sandy loam soil. The plants were given Hoagland's solution (10 mM KNO_3) at weekly interval. Third and fourth leaves of 20–25-day old seedlings were selected for the study. The leaves were cut under water and fed with various treatment solutions via the transpirational stream at a light intensity of

$800 \, \mu E \, m^{-2} s^{-1}$. The pre-treatment of the leaves with either $NO_3^-(10 \, mM)$ or glycine (10 mM) was done for 3 h. The concentration of MSO throughout the experimentation was 2·5 mM. Ammonia in the leaf extracts was determined according to Kumar *et al* (1984).

NR activity was assayed according to Klepper *et al* (1971). NiR activity was determined following an *in vivo* procedure developed in our laboratory. Fine slices of leaves (<1 mm) were suspended in a medium containing 300 μmol potassium phosphate (pH 6·9), 3 μmol methyl viologen and 0·5 μmol sodium nitrite. Reaction was initiated by the addition of 20 μmol of sodium dithionite prepared in 0·05 mM phosphate buffer. After incubation for 15 min at 33°C, the reaction was stopped by vigorous agitation of the reaction mixture. Nitrite disappeared was estimated by comparing with a zero time control in which reaction was stopped immediately after the addition of sodium dithionite. GS activity in the leaves was estimated following the procedure of Mohanty and Fletcher (1980). NADH–GDH activity was assayed in the crude mitochondrial fractions, as described by Mohanty and Fletcher (1980).

3. Results and discussion

MSO is a potent inhibitor of GS and ammonia assimilation (Kumar *et al* 1983). Treatment of barley leaves with 2·5 mM MSO resulted in the accumulation of ammonia (figure 1). Pre-treatment of the leaves with nitrate enhanced the rate of ammonia accumulation. This could be due to an increased flux of nitrogen via nitrate assimilation pathway as a result of NR induction (Beevers and Hageman 1983). Similarly, pre-treatment of the leaves with glycine considerably elevated the rate of ammonia accumulation. Ammonia is released during glycine oxidation in mitochondria under photorespiratory conditions (Singh *et al* 1985) and the flux of ammonia through the glycolate pathway is 8–10 times more than that occurring via nitrate assimilation route (Keys *et al* 1978). At the end of 2 h MSO treatment the ammonium level was about 11·2 μmol/g fresh weight of the leaves (nitrate-treated). This concentration was considered to be toxic enough to affect the processes of photosynthesis in the chloroplasts (Givan 1979). In the subsequent experiment,
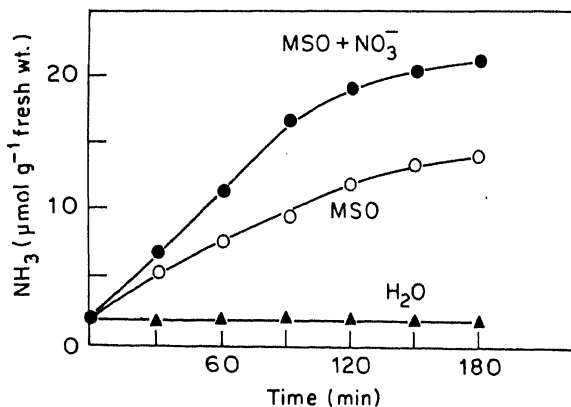
Figure 1. Effect of methionine sulphoximine on the accumulation of ammonia in barley leaves.

leaves treated with nitrate (10 mM) for 3 h were transferred to MSO and activities of the enzymes, NR, NiR, GS and NADH-GDH were assayed at different time intervals up to 24 h.

Table 1 shows the effects of MSO on the activities of the enzymes of nitrogen assimilation. GS activity is inhibited almost completely by 3 h. NR activity persisted in the leaves throughout the treatment period. However, there was a 50% decline at the end of 12 h. NiR activity was more resistant to MSO treatment. There was a decrease of only 22% at 24 h. NADH-GDH activity showed no perturbance consequent to MSO treatment. These results showed that the enzymes of nitrogen assimilation except GS, are more or less resistant to MSO treatment. NR depends on the supply of carbohydrates as the source of reducing power for its action (Beevers and Hageman 1983). Similarly, NiR derives its reducing potential from ferredoxin which is reduced during photosynthetic electron transport (Abrol *et al* 1983). The insensitivity of these enzymes to MSO treatment indicates that the processes of photosynthesis in the chloroplasts are not seriously affected as a result of the accumulation of ammonia. The decline in NR activity during the later stages of MSO treatment could be attributed to the limitation of the substrate, nitrate. This was supported by the observation that in nitrogen-starved cells of *Chlamydomonas reinhardii*, MSO inhibited NR activity. The activity was restored by exogenous supply of nitrate (Florencio and Vega 1983). It was also observed that MSO did not affect NiR activity.

GDH is considered to play a minor role in the assimilation of ammonia in higher plants (Kumar and Abrol 1990). The possible role of GDH in an ammonia detoxification process is supported by the finding that the enzyme is induced by high levels of ammonia (Barash *et al* 1973). Similarly, GDH activity increased during senescence, dark stress and proteolysis indicating its significance in situations of high ammonia levels. In the present study, we did not observe any change in GDH activity even after prolonged MSO treatment and we deduce that the enzyme is not involved in ammonia assimilation process. Cammaerts and

Table 1. Effect of MSO on the activities of the enzymes of nitrogen metabolism in the leaves of barley.

Enzyme	Time (h)				
	0	3	6	12	24
NR *in vivo*					
(μmol g^{-1} fresh wt. h^{-1})	2·00	1·94	1·36	1·08	1·05
	(100)	(97)	(68)	(54)	(52)
NiR *in vivo*					
(μmol g^{-1} fresh wt. min^{-1})	3·24	3·16	3·04	2·86	2·53
	(100)	(97)	(94)	(88)	(78)
GS					
(μmol γ-GHA g^{-1} fresh wt. min^{-1})	5·40	0·02	0·01	ND	ND
	(100)	(0·37)	(0·18)	(0)	(0)
NADH-GDH					
(μmol NADH mg^{-1} protein min^{-1})	14·16	13·78	14·23	14·03	13·61
	(100)	(97)	(100)	(99)	(96)

Values in parentheses indicate per cent change.
ND, Not detected.

Jacobs (1985) reported an increase in the level of NAD–GDH but not of NADH–GDH as a result of MSO treatment to *Arabiodopsis thaliana* seedlings. However, NADH–GDH in the roots was induced by MSO treatment. This suggested that GDH is probably involved in ammonia detoxification in the roots.

In conclusion, it can be stated that MSO-induced ammonia accumulation does not inhibit the activities of NR and NiR. The elevated levels of ammonia do not cause any increase in the activity of GDH and thus it may not play any role in ammonia assimilation in the leaves.

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Stability of pollen sterility in cytoplasmic-genetic male sterile lines in rice

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Abstract. On the basis of the shape and staining pattern of pollen grains, 22 cytoplasmic-genetic male sterile lines of 5 different cytosterility systems in rice could be classified into 4 categories, viz., unstained withered sterile, unstained spherical sterile, stained round sterile and stained round fertile. Using this system, the cytoplasmic-genetic male sterile lines were classified into 5 groups, characterised by relative frequency of the different classes of pollen grains.

These cytoplasmic-genetic male sterile lines were grown during 6 seasons (1984 and 1985 wet, 1986 and 1987 dry and wet) to study the stability of pollen sterility. Six cytoplasmic-genetic male sterile lines (V 20 A, Zhenshan 97 A, IR 54753 A, IR 54754 A, IR 54757 A and IR 54758 A) having wild abortive type cytoplasm were found to be stable for pollen sterility.

Keywords. Male sterile lines; pollen shape; stainability; stability.

1. Introduction

Cytoplasmic-genetic male sterility is a pre-requisite for the development of hybrid rice. Male sterility in rice could be due to abnormality at any stage from microsporogenesis to pollen maturation. An ideal cytoplasmic-genetic male sterile (CMS) line should have complete and stable pollen sterility for its utilisation in hybrid rice programme. The current study describes the results on various types of sterile pollen grains and stability of pollen sterility in dry and wet seasons of 22 CMS lines in rice.

2. Materials and methods

Pollen grains of 22 CMS lines of 5 cytoplasm sources were observed for pollen sterility under microscope. Eighteen CMS lines (V 20 A, Zhenshan 97 A, IR 54753 A, IR 54754 A, IR 54757 A, IR 54758 A, IR 46829 A, IR 46830 A, Er-Jiu-Nan 1 A, IR 48483 A, IR 54752 A, IR 54756 A, V 41 A, Madhu A, IR 46831 A, IR 46828 A, IR 46826 A, IR 46827 A) had wild abortive (WA) cytoplasm and one each from Gambiaca (i.e. Yar-Ai-Zhao A), T(N)1 (Pankhari 203 A), Chinsurah Boro-II (Wu 10 A) and *Oryza sativa* f. *spontanea* type (MS 577 A) cytoplasm. Spikelets from 10 panicles were collected at random from each CMS lines between 8.30–9.30 h during anthesis. Pollen grains from 4 mature anthers/spikelet were collected from 5 randomly selected spikelets/panicle from each CMS lines and were stained in 1% lugol's iodine solution. More than 1000 pollen grains from each line were examined with a magnification of X120. The number of sterile and fertile pollen grains were counted and the stability was expressed in percentage. Thirteen CMS lines were grown in 6, two in 5 and rest 7 in 3 successive seasons to study the stability of pollen sterility.

3. Results and discussion

The pollen grains were grouped into 4 categories based on the shape and staining pattern following the classification of Chaudhury *et al* (1981)—unstained withered sterile (UWS), unstained spherical sterile (USS), stained round sterile (SRS) and stained round fertile (SRF) (figure 1). UWS and USS pollen grains did not take stain due to the absence of starch while SRS and SRF pollen grains were stained due to the presence of starch grains. SRS pollen grains were smaller in size, stained light brown with rough surface and loosly packed starch grains. SRF pollen grain were bigger in size, took dark blue or black stain with smooth surface and were fertile. The panicles of the CMS line Wu 10 A having maximum (65%) SRS pollen grain when bagged before pollination did not set seed while the panicles of the CMS line IR 46826 A having only 30% SRF pollen grains showed about 25% seed set on selfing.

No seed set was observed when the pollen of Wu 10 A were dusted on the stigma of V 20 A CMS line. There was seed set when V 20 A was pollinated with the SRF type pollen grains of Wu 10 B. While screening for maintainers and restorers for various CMS lines, it was observed that Jhona-349 maintained completely the sterility of Wu 10 A (65% SRS pollen) and the F_1, Wu 10 A × Jhona-349 showed about 95–99% SRS type pollen grains. This indicated that the SRS type pollen grains were completely sterile although they were stained lightly due to loose packing of starch in the pollen grains.

Relative frequency of each category of pollen grain was expressed as percentage
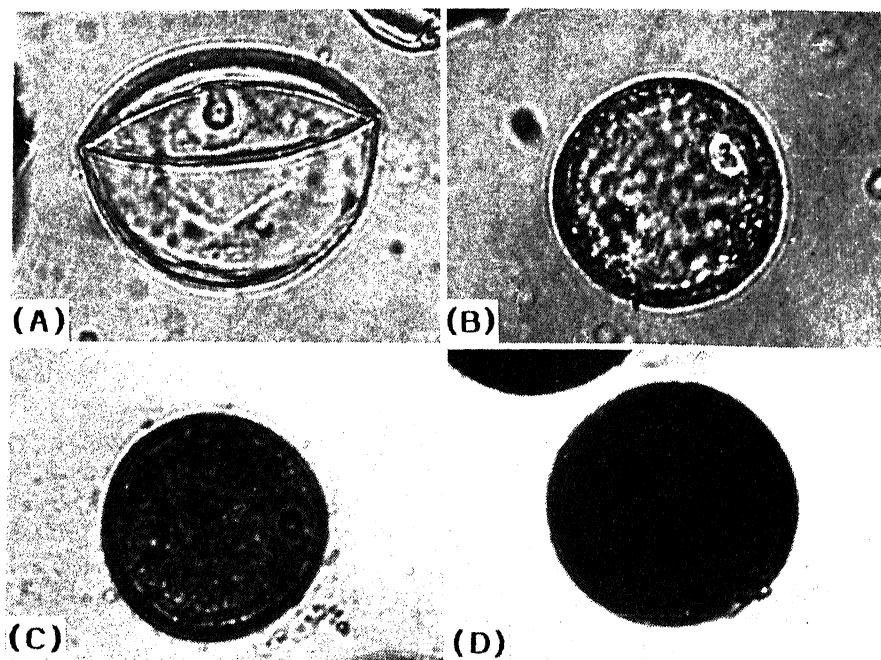
Figure 1. Types of pollen grain in CMS lines (× 600). (A) Unstained withered sterile. (B) Unstained spherical sterile. (C) Stained round sterile. (D) Stained round fertile.

of the total number of pollen grains examined for each CMS line. Depending on the predominant class of pollen grains, the 22 CMS lines could be classified into 5 groups (table 1).

Based on this classification of stained pollen grains Chaudhury *et al* (1981) classified 5 CMS lines from 3 different sources into 3 groups. Virmani and Edward (1983) reported that most of the pollen grains of MS 577 A (*O. sativa* f. *spontanea* type) were stained and sterile. However, in the present study, only 4·7% of the pollen grains of MS 577 A were found to be stained and sterile indicating that the stainability is perhaps, influenced by the environment.

Some of the CMS lines were grown from 1984–1987 in wet and dry seasons at Cuttack to check the stability of pollen sterility. Eight CMS lines of WA type (V 20 A, Zhenshan 97 A, IR 54752 A, IR 54753 A, IR 54754 A, IR 54756 A, IR 54757 A and IR 54758 A) were found to be complete sterile and remained unaffected by seasonal influences (table 2). The CMS lines derived from the WA cytosterility system were reported to be most stable for their complete or nearly complete pollen sterility over environments in China (Lin and Yuan 1980) and at the International Rice Research Institute (IRRI), Philippines (Virmani *et al*., 1981;

Table 1. Frequency of different categories of (sterile and fertile) pollen grains in 22 CMS lines (1986 wet season).

CMS lines	Different categories of pollen grains (%)			
	UWS	USS	SRS	SRF
Group-1				
V 20 A	98·5	1·5	—	—
Zhenshan 97 A	96·6	3·4	—	—
IR 54753 A	91·4	8·3	—	—
IR 54754 A	96·5	3·5	—	—
IR 54757 A	94·9	5·1	—	—
IR 54758 A	97·8	2·2	—	—
IR 46829 A	93·4	6·6	—	—
IR 46830 A	93·8	6·2	—	—
Er-Jiu-Nan 1A	91·7	8·3	—	—
IR 48483 A	95·2	4·8	—	—
Yar-Ai-Zhao A	91·8	8·2	—	—
Group-2				
IR 54752 A	93·4	2·8	3·8	—
IR 54756 A	91·9	3·5	4·6	—
V 41 A	92·3	2·6	5·1	—
MS 577 A	88·8	6·5	4·7	—
Group-3				
Madhu A	92·0	4·2	—	3·8
IR 46831 A	95·3	3·7	—	1·0
IR 46828 A	91·8	3·2	—	5·0
IR 46826 A	68·6	1·4	—	30·0
IR 46827 A	82·3	2·7	—	15·0
Group-4				
Pankhari 203 A	37·3	41·6	21·1	—
Group-5				
Wu 10 A	30·0	5·0	65·0	—

Table 2. Pollen sterility percentage of 22 CMS lines during different seasons from 1984 wet to 1987 wet seasons.

CMS lines	Pollen sterility (%)					
	1984 Wet	1985 Wet	1986 Dry	1986 Wet	1987 Dry	1987 Wet
V 20 A	100	100	100	100	100	100
Zhenshan 97 A	—	100	100	100	100	100
IR 54753 A	—	—	—	100	100	100
IR 54754 A	—	—	—	100	100	100
IR 54757 A	—	—	—	100	100	100
IR 54758 A	—	—	—	100	100	100
IR 46829 A	97	98	100	100	98	100
IR 46830 A	98	99	100	100	97	100
Er-Jiu-Nan 1 A	—	98	99	100	99	99
IR 48483 A	90	90	93	100	99	100
Yar-Ai-Zhao A	100	100	90	100	97	100
IR 54752 A	—	—	—	100	100	100
IR 54756 A	—	—	—	100	100	100
V 41 A	99	98	95	100	96	95
MS 577 A	—	—	—	100	98	99
Madhu A	—	95	98	96	99	100
IR 46831 A	94	97	99	99	99	99
IR 46828 A	92	95	90	95	94	96
IR 46826 A	90	75	75	70	81	75
IR 46827 A	95	90	80	85	83	81
Pankhari 203 A	100	100	99	100	99	100
Wu 10 A	99	100	99	100	99	100

Yuan and Virmani 1986). Out of the 11 cytosterile lines maintained at IRRI, only 7 (V 20 A, Zhenshan 97 A, Er-Jiu-Nan 1 A, V 41 A, Yar-Ai-Zhao A, Pankhari 203 A and Wu 10 A) were reported to be relatively stable for pollen sterility (Virmani and Edward 1983). In the present investigation, the CMS lines IR 46826 A and IR 46827 A did not show complete pollen sterility in any of the seasons, while V 41 A, Er-Jiu-Nan 1 A, IR 46828 A, IR 46829 A, IR 46830 A, IR 46831 A, IR 48483 A, Madhu A, Yar-Ai-Zhao A, Pankhari 203 A, Wu 10 A and MS 577 A were unstable for the expression of sterility over seasons (table 2). Based on the frequency of different categories of pollen grains in different CMS lines and expression of pollen sterility over seasons, it could be inferred that the CMS lines V 20 A, Zhenshan 97 A, IR 54753 A, IR 54754 A, IR 54757 A and IR 54758 A derived from WA cytosterility system were the ones that were most stable at Cuttack.

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Changes in the phenolic contents of rice cultivars towards air pollution exposure

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Abstract. Three cultivars of rice (*Oryza sativa* L.) were exposed to the polluted environment of a fertilizer plant to evaluate the changes in the phenolic contents due to air pollution. Two soil N regimes under normal watering and water stressed (half-field capacity) conditions were imposed on 30-day old plants. Controls were maintained 10 km away from the factory in an unpolluted area using the same cultural practices. As the main effects, air pollution decreased the levels of phenolic content at 55 days and increased at 70 days. The cv. GR 3 appeared to accumulate high phenolic contents at the later stage while cv. CO 43 decreased the same. The cv. TKM 9 showed the similar pattern as that of cv. GR 3. The interaction of cultivar with nitrogen and cultivar with water produced no significant effect. It was concluded that the main effect of air pollution appeared to be the increased amount of phenolic contents towards growth and the accumulation was high only in tolerant cultivars.

Keywords. *Oryza sativa*; air pollution; phenols.

1. Introduction

Sulphur dioxide, oxides of nitrogen and ozone are some of the major pollutants in the atmosphere. Ammonia and fluoride have also been found often in industrial atmospheres especially in the vicinity of synthetic fertilizer factories (Harrison and McCartney 1979; Anbazhagan *et al* 1989a). All are phytotoxic to varying extents. Among the metabolic responses of plants to air pollution, changes in free amino acid concentrations and activities of enzymes involved in amino acid metabolism have been well established (Jaeger and Klein 1977; Wellburn 1987). To assess these responses some of the reliable and sensitive methods have been reviewed (Darral and Jaeger 1984) and such methods must reflect their utility in the assessment of the levels of pollutants in a mixed form which occur in the field condition. In the O_3 sensitive cultivars of *Arachis hypogea* L. accumulation of high phenolic contents were reported as compared to tolerant plants (Howell 1974).

Our recent reports on the performance of 3 cultivars of rice to the conditions near a fertilizer plant showed cultivar differences in sensitivity to air pollutants (Anbazhagan *et al* 1989a) and studies on the same cultivars to artificial fumigation of SO_2, NH_3 and NO_2 singly and in mixtures showed the accumulation of free proline as an indicator of stress tolerance (Anbazhagan *et al* 1988). It has also been reported that sensitivity to air pollution can be predisposed by modifying nitrogen nutrition in rice (Anbazhagan *et al* 1989b), and water stressed tomato plants are less sensitive to air pollutants due to induction of stomatal closure by water stress which in turn will modulate the effect of gaseous pollutants (Khatamian *et al* 1973). We describe here the accumulation of phenolic content in rice plants after exposure to the conditions near a fertilizer plant under modified nitrogen nutrition and water status to evaluate their potential usefulness as a cumulative pollution stress index.

2. Materials and methods

The experiment was performed with rice plants (*Oryza sativa* L.) cvs. CO 43, GR 3 and TKM 9. Rice seeds were procured from the Tamil Nadu Agricultural University, Coimbatore (cvs. CO 43, TKM 9) and the Main Rice Research Station, Navagam (cv. GR 3). Three-day old pregerminated seeds were sown in polythene pots containing 8 kg of soil. The pots were divided between two locations, a polluted site (near Gujarat State Fertilizer Company Ltd., which emits SO_2, NH_3, NO_2 and F as major pollutants) and an unpolluted 'control' site, at each of which the factorial combinations of 3 cultivars, two water regimes and two doses of nitrogen were tested in a factorial randomised design with 3 replicates. The mineral composition of the water extract of the test soil was: pH 6·3, electrical conductivity 0·6 mmhos/cm³, sodium 1·6, nitrogen 12·5, potassium 1·08, phosphorous 33·6, calcium 4, magnesium 2·7, chloride 0·55, bicarbonate 21, and sulphate 1·16 m eq/l. The standard procedures followed for the determination of concentrations of air pollutants were as described elsewhere (Anbazhagan *et al* 1989a) and the levels of pollutants at both the sites are given in table 1. There were no altitudinal variations between the sites. The mean temperature maximum was 34·7°C, minimum 23·3°C, total rainfall 70·9 mm, mean wind speed 5·6 km/h and relative humidity 66·7% during the growth period.

Ten days after seeding, plants were thinned to 4 equidistant plants per pot. Two water regimes were imposed from day 30 onwards: (i) daily watering to field capacity and (ii) 50% water stressed (50% field capacity maintained till the harvest by weight method). Two doses of nitrogen, as urea, were tested; small nitrogen (96 mg N kg⁻¹ dry soil applied in two equal amounts during growth) and large nitrogen (224 mg N kg⁻¹ dry soil applied in 3 equal amounts during growth).

At the age of 55 and 70 days the total phenolics were extracted by grinding 50 mg of dry leaf material using a chilled mortar and pestle with a sample of chilled 80% (v/v) ethanol. The homogenate was centrifuged at 5000 *g* for 15 min. The supernatant was collected and the residual pellet was extracted twice again. A sample of the ethanolic extract was diluted with distilled water to 8·5 ml and after adding 0·5 ml of Folin-phenol reagent, the contents were mixed well as per the method of Swain and Hillis (1959). Three min later 1 ml of saturated sodium carbonate solution was added and the mixture was allowed to stand for 1 h after thorough mixing. Using a Carl Zeiss colorimeter the optical density was measured

Table 1. Concentrations of some pollutants at the control and polluted sites during growth of rice plants.

Parameters	Control site	Polluted site
Sulphation rate (mg SO₄/100 cm²/d⁻¹)	0·22 (0·12–0·26)	0·95 (0·78–1·07)
NO₂ (μg/m³)	Negligible	25·20 (19·0–38·0)
NH₃ (mg/m³)	Negligible	1·64 (1·54–1·76)
F (μgF/cm²/month)	Negligible	0·40 (0·33–0·52)

Numbers in parentheses represent minimum and maximum values.

at 725 nm and the phenolic contents were determined with chlorogenic acid as the standard. The data from 3 replicates were analysed by analyses of variance (ANOVA).

3. Results

The effects of the environment on the accumulation of phenolic contents of rice plants at 55 and 70 days (table 2) were significant. The independent effect of cultivar was evident only at the later stage. There was no first order or second order interaction of the factors cultivar, nitrogen, water and environment. However, interactive effect of all the 4 factors ($C \times N \times W \times E$) were evident significantly as indicated by the mean square values of the ANOVA.

In polluted air, the accumulation of phenolic contents appeared to decrease at 55 days but increased by 70 days (table 3). A significant decrease of 21% in the cultivar GR 3 was evident in 55-day old plants. At 70 days a decrease of 15% was observed in CO 43 while it was increased significantly by 34 and 19% in cvs. GR 3 and TKM 9, respectively. Interaction between cultivar and applied nitrogen ($C \times N$) showed a significant decrease in the phenolic contents in the cv. CO 43 (table 4). There was no other significant effects due to $C \times N$ and $C \times W$ (table 4).

The interaction of small amount of applied nitrogen with cultivar and environment showed a significant increase in the phenolic contents of cv. CO 43 at 55 days but decreased at 70 days (table 5). The accumulation pattern was opposite in the case of GR 3. The cv. TKM 9 showed an increased amount at 70 days. However, under the application of large amount of N, only decrease in cv. CO 43 at 55 and 70 days was observed while cv. GR 3 produced a less amount at 55 days

Table 2. Mean square values from the analysis of variance of rice response variables, on the phenolic content.

Source	df	Age in days	
		55	70
Replication	2	1·88	0·79
Cultivar (C)	2	4·34	22·53*
Nitrogen (N)	1	0·18	15·89
Water (W)	1	0·08	31·73
Environment (E)	1	9·68**	108·03**
$C \times N$	2	0·18·	1·34
$C \times E$	2	0·63	12·32
$C \times W$	2	0·08	6·22
$W \times N$	1	0·01	5·06
$W \times E$	1	0·01	1·93
$E \times N$	1	0·49	1·56
$C \times N \times E$	2	2·22	30·12
$C \times N \times W$	2	0·77	23·12
$C \times W \times E$	2	1·20	37·33
$N \times W \times E$	1	2·65	6·89
$C \times N \times W \times E$	2	5·63**	86·52**
Error	46	1·04	10·08
Total	71		

Levels of significance: *$P = 0.05$; **$P = 0.01$.

Table 3. Interaction of cultivar and environment in relation to phenolic contents (mg/g dry wt.) of rice grown near to (P) or distant from (C) a fertilizer plant.

Cultivar	55 days		70 days	
	C	P	C	P
CO 43	8·75	8·85	20·95	17·8**
GR 3	9·65	7·65**	17·95	24·0**
TKM 9	9·60	9·30	19·15	22·85**
Mean	9·33	8·60*	19·2	21·55**

Significant difference over the control: *$P = 0.05$; **$P = 0.01$.

Table 4. Interaction of cultivar with applied nitrogen and with water regimes in relation to the phenolic contents (mg/g dry wt.) of rice.

	55 days		70 days	
Cultivar × applied nitrogen				
	Small N	Large N	Small N	Large N
CO 43	9·35	8·25*	18·95	19·60
GR 3	9·65	9·00	20·05	19·45
TKM 9	9·40	9·50	21·10	20·50
Cultivar × water regimes				
	Full field capacity	50% field capacity	Full field capacity	50% field capacity
CO 43	8·85	8·75	18·15	20·6
GR 3	8·75	8·50	23·05	18·45
TKM 9	9·20	9·70	21·65	20·35

*Significant difference at $P = 0.05$.

Table 5. Interaction of cultivar, applied nitrogen and environment on the phenolic contents (mg/g dry wt.) of rice grown near to (P) and distant from (C) a fertilizer plant.

Amount of nitrogen	Cultivar	55 days		70 days	
		C	P	C	P
Small	CO 43	8·7	10·0**	20·9	17·0**
	GR 3	9·2	7·4**	17·5	26·6**
	TKM 9	9·2	9·6	18·3	23·9**
Large	CO 43	8·8	7·7**	21·0	18·6**
	GR 3	10·1	7·9**	17·5	21·4**
	TKM 9	10·0	9·0**	20·0	21·8*

Significant difference over the control: *$P = 0.5$; **$P = 0.01$.

and significantly high amount at 70 days. The cv. TKM 9 also showed a similar pattern of accumulation.

The interaction of imposed water regimes with cultivar and environment produced less accumulation of phenolics in cv. GR 3 at 55 days and higher amount

Table 6. Interaction of water stress, cultivar and environment on the phenolic contents (mg/g dry wt.) of rice grown near to (P) and distant from (C) a fertilizer plant.

Water regimes	Cultivar	55 days		70 days	
		C	P	C	P
Full field capacity	CO 43	8·8	8·9	19·5	16·8**
	GR 3	9·6	7·8**	19·6	26·5**
	TKM 9	9·4	9·0	22·3	21·0
50% field capacity	CO 43	8·7	8·8	22·4	18·8**
	GR 3	9·6	7·5**	15·4	21·5**
	TKM 9	9·8	9·6	16·0	24·7**

Significant difference over the control: *$P = 0.05$; **$P = 0.01$.

than the control at 70 days at both water levels (table 6). At 70 days the content was significantly decreased in cv. CO 43. On the otherhand cv. TKM 9 showed increment in phenolics accumulation in the polluted environment at 70 days only under 50% water stress.

4. Discussion

Our recent reports on the 3 cultivars of rice showed cv. GR 3 to be tolerant, cv. TKM 9 as intermediate and cv. CO 43 as sensitive to the conditions near a fertilizer plant (Anbazhagan *et al* 1989). Moreover, the above rice cultivars when exposed to artificial fumigation of SO_2, NH_3, NO_2 pollutants singly and in combinations under 3 N levels accumulated higher amount of free proline as an indicator of stress tolerance (Anbazhagan *et al* 1988). Biochemically resistance might result from a multivalent derepression of isoflavonoid biosynthesis (Keen *et al* 1972) in plants. Though the cv. CO 43 showed no change in the accumulation of phenolic content at 55 days, it showed reduction at later stage. On the other hand cvs. GR 3 and TKM 9 accumulated higher amounts of phenolics at the later stage probably due to their tolerant nature, as indicated by their yield pattern (Anbazhagan *et al* 1989).

It was interesting to note that there was no significant interactive effect due to environment with cultivar (C × E), as apparent from the mean squares of ANOVA of the phenolic contents. However, the independent effect of environment turned out to be highly significant. The above situation confirms the discussion of Oshima and Bennett (1979) on the use of treatments (factors) for air pollution studies, where the studies did not require a statistical significance as a pre-requisite and could be applied regardless of the results of overall analysis of variance. It is because that partitioning of the degrees of freedom and treatment sum squares for evaluating the main and interacting effects of factors, individually and in combination, has the advantage of using the power of the single degree of freedom comparison rather than the multiple range comparison test, which is less efficient as the number of factors increases. Since there was no remarkable significant effects due to N and W as depicted by mean squares (table 2) the effects appeared to be only due to environment under C × E.

Nouchi and Odaira (1973) noted that ozone led to the accumulation of anthocyanin cyanidin in morning glory plants and Howell (1970) reported the

accumulation of several fluorescent compounds in green beans after exposure to ozone, one of which was identified as caffeic acid. Moreover, the sensitive cultivars of *Arachis hypogea* appeared to accumulate a high phenolic content as compared to plants tolerant to O_3 pollution (Howell 1974). However, in the present study the changes in the phenolic contents at different stages of cultivar growth could have been due to the cultivar responses to the mixed polluted environment. The cultivars which accumulated higher phenolics at later stage showed relationship with their tolerance nature. This may be due to a variety of physiological and morphological characters that are themselves influenced by genotype and environment (Roose *et al* 1982).

It can be concluded that the main effect of air pollution appeared to be the increased amount of phenolic contents towards growth. Nevertheless, the accumulation was high only in tolerant rice cultivars.

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Factors affecting germination of *Peronospora parasitica* in radish

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Abstract. Oospores of *Peronospora parasitica* are found to be dependent on
environmental factors such as temperature, light, pH of the medium and age of oospores.
Optimum temperature of 23°C is required for their germination. Drying and chilling had
no marked effect on oospore germination. At pH 7·5, 42% germination was recorded while
at pH 4·5 only 1% of oospores germinated. Oospore germination increased with increase
in their age.

1. Introduction

Germination of oospores of many downy mildew pathogens, have been tried and
few workers have succeeded in their attempts (Weston and Uppal 1932; Safeeulla
1976; Shetty and Safeeulla 1980). Shaw (1970), stated that only when we know how
to assure germination of any collection of oospores, we can have a complete
confidence in infection experiments involving oospores. Oospores of *Peronospora
parasitica* (Pers. ex. Fr.) Fr. in radish have been germinated in agar medium,
mixture of host root exudate and soil extract (Jang 1989). Since a detailed study of
the environmental factors influencing germination of this pathogen in radish is not
available, the present investigation is undertaken.

2. Materials and methods

2.1 *Effect of temperature*

Oospores (400) maintained in 2% agar were incubated at different temperatures
viz., 5, 10, 15, 16, 20, 23, 25, 28, 30 and 35°C for a period of 12, 48 and 72 h
respectively. Germination of oospores was noted down at the end of each period.

2.2 *Effect of drying and chilling*

Oospores suspension was gradually air-dried by transferring, at 24 h intervals, to
higher temperatures ranging from 16–40°C (4 degrees intervals). Similar oospores
suspension was transferred from 16°C to chilling temperatures up to −5°C at 24 h
intervals, with 4 degrees intervals between each treatment. After each experimental
set up percentage of oospore germination was counted for every 400 oospores used.

2.3 *Effect of light*

Oospores (400) maintained in 2% agar were incubated at 23±1°C in continous

light, continous darkness and alternate light and darkness for a period of 12, 48 and 72 h respectively and oospores germination noted down at the end of each period.

2.4   *Effect of pH*

One M HCl or NaOH was added to adjust pH level varying from 4·5–9·5 at 12, 48 and 72 h respectively. Four hundred oospores were used per pH level and germination percentage was calculated.

2.5   *Effect of age*

Harvested oospores were stored under laboratory conditions for varying periods viz., 1 day, 1 week, 2 weeks, 6 months, 1 year and 2 years. Such oospores were incubated at $23 \pm 1°C$ in 2% agar for a period of 12, 48 and 72 h respectively. Freshly harvested young oospores were subjected to germination. Four hundred oospores were used for each treatment and at the end of each period germination percentage was counted.

3.   Results

3.1   *Effect of temperature*

Oospores maintained on 2% agar germinated at all temperatures ranging between 13–30°C incubated for 72 h. Optimum temperature favourable for oospore germination under these conditions was 23°C. No germination was seen at temperature below 13°C and above 30°C.

3.2   *Effect of drying and chilling*

Drying and chilling had no effect on germination of oospores. No germination was seen at −5°C and at 40°C.

3.3   *Effect of light*

Light was observed to play an important role in germination of oospores. Germination was very low when oospores were subjected to continous light (2%) and continous darkness (2, 7%) for 12, 48 and 72 h, respectively. In alternate light and darkness under similar conditions, oospores germinated with higher percentage. Maximum germination (35%) was observed from those oospores subjected to alternate light and darkness at $23 \pm 1°C$ for 72 h.

3.4   *Effect of pH*

Out of 400 oospores counted per pH level, percentage of germination was highest (42%) at pH 7·5 and least at pH 4·5 (1%). Germination percentage showed slight increase from pH 4·5–7·5, thereafter decreased up to pH 9·5.

3.5 *Effect of age*

Germination of oospores was dependent on their age. Young oospores (freshly harvested) germinated (1%) but higher percentage was obtained when the oospores were stored for varying periods from time of harvest. Oospores germination increases with age. One year old oospores gave 41% of germination, however, longer periods of storage reduce germination and 2 years old oospores did not germinate.

4. Discussion

Earlier reports suggest that oospores of downy mildew pathogen have the ability to germinate given a specific temperature range, light regime, humidity and even pH concentration (Tasugi 1933; Kaveriappa 1973; Safeeulla 1976). From this study, it is evident that the highest percentage of oospore germination occurred at all temperatures ranging between 13–30°C. Light is another factor which is seen to influence oospore germination. A good percentage of germination is obtained when the oospores are subjected to alternate light and darkness. Our results would certainly help to understand the downy mildew disease in radish from epidemiological point of view. Even the pH of the oospore suspension is seen to be an important factor initiating oospore germination of this pathogen. An oospore suspension of pH level 7·5 is seen to be congenial for oospore germination in this study, emphasising its importance in any experiments involving oospore germination and infection percentage from inoculum suspension. Tasugi (1933) observed an increasing percentage of germination of *Sclerospora graminicola* at pH range of 2·9 and 3·1, which decreased when pH was raised to 9. Although aging improved germination of oospores in *P. parasitica*, long periods of storage did not help this process as revealed in this study. Germination increased with age of oospores up to the first year and thereafter decreased in the second year. Safeeulla (1970), reported 5–10% oospore germination in *Sclerospora sorghi* with one year old oospores. He also suggested that oospores of *S. macrospora* should undergo a period of storage in the laboratory for about 8 weeks before they germinate.

Germinating oospores of *P. parasitica* under laboratory conditions successfully should stimulate further research work of the host and the pathogen.

Acknowledgement

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Production and viability of *Peronospora parasitica* in radish

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Abstract. In *Peronospora parasitica* the inoculum load is found in the form of oospores in the leaf and seed tissues of radish. Out of 400 seeds tested, ·10% showed the presence of oospores in the pericarp and 0·1% in the embryo. The 2,3,5-triphenyltetrazolium chloride test is a quick method of determining the viability of the oospores. Viability of oospores based on infection capacity after storage, though a long process, is effective and reliable. Results of *in vitro* and *in vivo* experiments show that the oospores need natural weathering, under field conditions, for a period of one year for maximum infection in radish and those stored for two years under the same conditions, has an adverse effect on their infection capacity. Infection capacity was higher among oospores exposed to weathering than those retained in laboratory.

Keywords. *Raphanus sativus*; *Peronospora parasitica*; oospores production; viability.

1. Introduction

Peronospora parasitica (Pers. ex. Fr.) Fr. causes downy mildew disease in *Raphanus sativus* L. (Baudys 1928). The inoculum occurs as mycelium in the host tissues (Baudys 1928; Ramsey *et al* 1954). However, the inoculum as oospores has not been documented in radish. The present study aims at unravelling details on the production of oospores and their viability.

2. Materials and methods

2.1 *Production of oospores*

Susceptible cultivar of radish (Japanese white) was sown in downy mildew nursery at Mysore. Infector rows were sown 2 weeks earlier to testar cultivars. When downy mildew disease appeared, oospores production was estimated at different stages viz., leaf, flowering and seed setting stages.

Maceration technique (Shetty *et al* 1978) was followed to detect the oospores in leaf tissues from the first pair to the tenth pair. The same technique was used to detect internally borne oospores in seeds (400 seeds were used for each treatment).

2.2 *Viability of oospores by triphenyltetrazolium chloride test*

Seeds and leaf tissues containing oospores were soaked in water for 12 h and then kept in different tubes containing 1% triphenyltetrazolium chloride (TTC) solution of pH 7. Such treated tissues were incubated at 30°C for 48 h in darkness and observed under the microscope. Based on the colour reaction, the oospores were

judged as viable or non-viable. Two susceptible cultivars were used to provide the oosporic materials at leaf, flowering and pod stages.

2.3   *Viability of oospores based on their age and percentage of infection after storage*

Dried powdered leaf tissues containing oospores were mixed with sterile garden soil and kept in dry small bags made of cheese cloth. Fifteen such bags were prepared, 5 of them retained in the laboratory, 5 of them kept in Downy Mildew Research Laboratory field on the surface of soil and the rest placed 15 cm below the soil level and covered by the same scooped soil. These bags were left as such for 1 and 2 years after which they were tested for viability.

Susceptible radish seeds were sown and number of seedlings grown and infected with such treated oospores were counted. Newly formed oospores mixed with garden soil served as control.

To determine the infectivity of oospores in soil, radish seeds were sown in pots containing sufficient oosporic materials mixed with garden soil and kept in green house. One month later, after recording the number of systemically infected seedlings, plants were pulled out before oospore formation and fresh lots of seeds were sown. After each month one crop was raised in each pot in which oospore inoculum was added once.

3.   Results

3.1   *Production of oospores*

Oospores were formed in leaf tissues (figure 1) and were also detected in the pericarp (figure 2) and embryo (figure 3). Oospores production in leaves increased from fourth (2%) to the tenth pair (15·9%). At the flowering and pod stage, the leaves showed higher percentage of oospores production (16·7 and 50% respectively). Mature seeds (10·1%) showed the presence of oospores in the pericarp, 0·1% in embryo region and no oospores were located in the endosperm.

3.2   *Viability of oospores by TTC test*

In the tetrazolium test oospores showing red colour in the cytoplasmic region were considered viable and non-viable oospores did not take any stain. Percentage of viable oospores was more in Japanese white than the other susceptible cultivar at all the stages (table 1).

3.3   *Viability of oospores based on their age and percentage of infection after storage*

Oospores viability was seen to be related to their age, since oospores stored for 1 and 2 years, respectively, showed variation in the percentage of infection (table 2). Maximum infection percentage was obtained with 1 year old oospores. There was a gradual reduction in infection percentage from first to second year. Oospores were very highly infective in the first year, thereafter their infection capacity decreases.
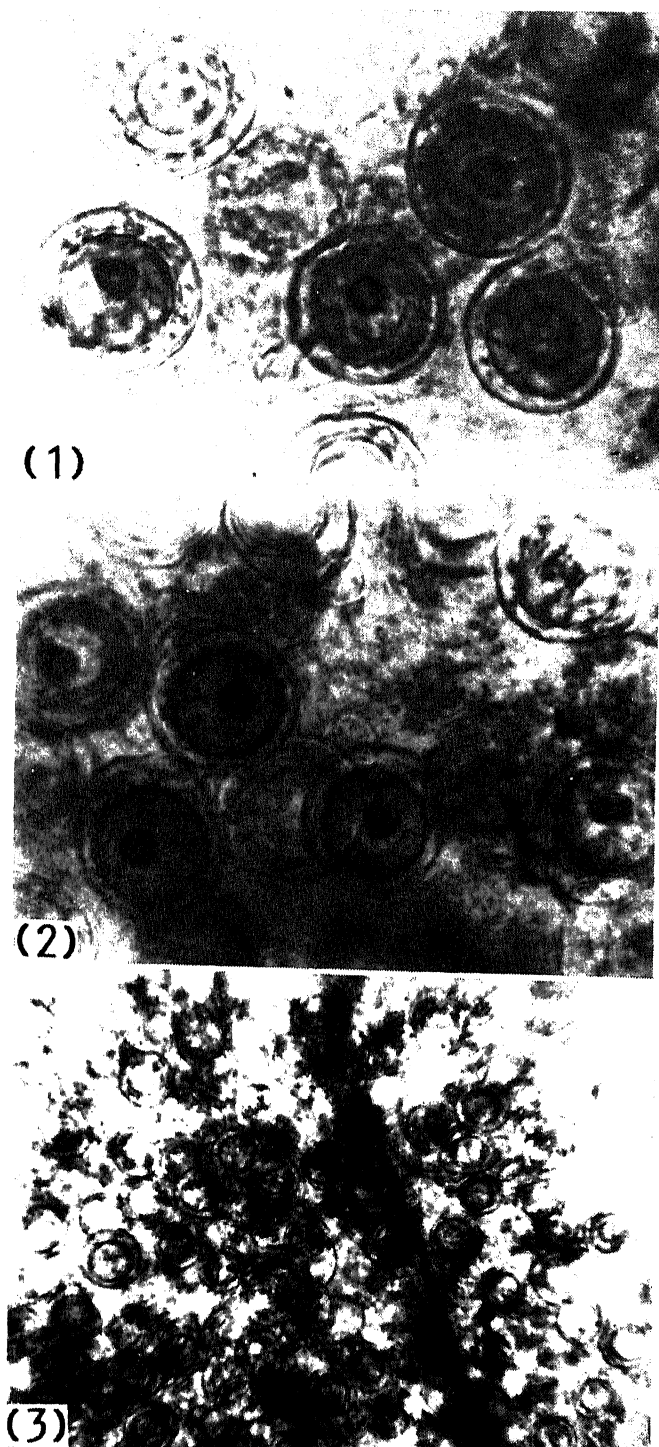
Figures 1–3. Oospores of *P. parasitica* in radish. **1.** Leaf tissues (× 1000). **2.** Pericarp region (× 1000). **3.** Embryo (× 200).

Table 1. Viable oospores of *P. parasitica* as tested by TTC.

Cultivars	Growth stages	Viable oospores (%) (based on 400 oospores count)	
		Leaf	Seed
Arka nishant	Flowering stage with immature seeds	2·5	2·2
Japanese white	–do–	3·9	4·0
Arka nishant	Flowering stage with mature seeds	10·5	14·5
Japanese white	–do–	15·1	16·2
Arka nishant	Pod stage with mature seeds	16·0	16·8
Japanese white	–do–	20·2	21·1

Table 2. Effect of age on oospore viability of *P. parasitica*.

	Age in years	
	1	2
Laboratory[a]		
Seedlings infected/grown	105/400	40/400
Infected plants (%)	30·75	15·50
Exposed to weathering[a] on the level of soil		
Seedlings infected/grown	410/450	205/460
Infected plants (%)	85·90	47·10
Below soil (15 cm)		
Seedlings infected/grown	440/455	210/460
Infected plants (%)	90·50	59·20
Control[b]		
Seedlings infected/grown	250/410	255/415
Infected plants (%)	55·90	30·50

[a]Replicated thrice; [b]newly formed oospores.

However, infection was higher among oospores exposed to weathering than those retained in laboratory conditions.

Crops raised in pots under green house conditions became infected due to oospores inoculum added to soil before sowing (table 3).

4. Discussion

Oospores production is an important process in the sexual stages of many plant pathogens and these oospores in most of the cases, form the primary source of inoculum. The present investigation reveals oospores formation in infected plants from fourth pair of true leaves and agrees with that of McMeekin (1960), who observed abundant oospores in necrotic leaves of *Brassica oleracea*. Oospores were not produced at cotyledons stage of radish seedlings which is contradictory to the observations of McMeekin (1960). Production of abundant oospores are noticed at the pod stage in the leaf tissues. Sansome and Sansome (1974) suggested that since both *Albugo candida* and *P. parasitica* commonly occur together in some Crucifers,

Table 3. Per cent infected plants in soil infested with oospores of *P. parasitica*.

Date of[a] sowing	Date of observation	Infected plants (%)
1st May 1983	20th May 1983	82·5
10th June 1983	24th June 1983	95·2
5th July 1983	28th July 1983	85·0
1st Aug. 1983	20th Aug. 1983	83·1
9th Sept. 1983	27th Sept. 1983	79·5
10th Oct. 1983	24th Oct. 1983	78·1
5th Nov. 1983	28th Nov. 1983	89·2
1st Dec. 1983	20th Dec. 1983	89·2
9th Jan. 1984	27th Jan. 1984	50·9
3rd Feb. 1984	26th Feb. 1984	15·1
11th March 1984	30th March 1984	03·0
2nd April 1984	22nd April 1984	05·0

[a]Oospores inoculum added only once before sowing; 400 seedlings raised in each crop.

cross stimulation of sexual reproduction may be possible. Further research work on 'interspecific stimulation' which may lead to abundant oospores formation of *P. parasitica* in radish is suggestive.

Oospores of *P. parasitica* in radish seeds could be an important source of primary inoculum particularly in the downy mildew free area. Such seeds should be given immediate importance since, the pathogen can move from an infested area to an uninfested one. The presence of oospores in radish seeds as revealed by our study, necessitates stringent seed testing before transporting seed materials to different places. Detection of oospores of the pathogen in seed tissue is also significant from epidemiological point of view of the disease in radish crops. Hence, control measure for spread of the disease among commercially grown radish crops, through seeds, should be given an immediate thought.

The tetrazolium test is found to be a quick method of testing the viability of *P. parasitica* oospores in radish in comparison with the viability test based on age of oospores and their infection capacity. Several workers have successfully tried the TTC method (Pathak *et al* 1978; Shetty *et al* 1978; Rao *et al* 1984).

Age of the oospores is seen to be an important factor which accounts for the viability of the oospores of *P. parasitica*. Percentage of infected plants decreased as age of the oospores increased from first to second year. The present findings are in confirmity with the observations made by Bhander and Rao (1967), who observed that oospores of *Sclerospora graminicola* were very highly infective in the first 3 years of storage and infectivity suddenly decreased during the fourth year. This is also true of *S. sorghi* (Kaveriappa 1973) and *S. graminicola* (Safeeulla 1976). Present study reveals that infection percentage by *P. parasitica* in radish is dependent on the age of the oospores, an important factor which decide their viability.

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Raft cultivation of *Gracilaria edulis* (Gmel.) Silva

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Abstract. The single-rope floating raft cultivation of *Gracilaria edulis* was tried at Krusadai Island for one year. In 3 harvests mean biomass annual yield of 4 kg (wet) m^{-1} was obtained which is the highest recorded for the alga. The raft cultivation of the alga at different levels has shown that maintaining the cultivation ropes at the top level will give better yield.

Keywords. *Gracilaria edulis*; single-rope floating raft; alga cultivation.

1. Introduction

Cultivation of seaweeds in the sea was termed 'marine agronomy' by Doty (1977). Among the economic marine algae only 11 genera are commercially cultivated to a certain extent in Japan, China, Philippines in the far east and these include *Porphyra tenera, P. yezoensis, P. haitanensis, Gelidiella acerosa, Gloiopeltis furcata, Eucheuma denticulatum, E. striatum, E. gelatinae, Gracilaria verrucosa, Laminaria japonica, Undaria pinnatifida, Macrocystis pyrifera, Monostroma grevillei, Entero-morpha intestinalis* and *Caulerpa racemosa*. Till now, only 4 major marine crop plants, viz., *Prophyra* spp. *Eucheuma* spp. *Laminaria japonica* and *Undaria pinnatifida* have actually been domesticated, for which the crop harvested exceeds that taken from wild populations (Tseng 1981a).

In India experimental field cultivation of agarophytes, *Gracilaria edulis* (Raju and Thomas 1971) by the long line rope method, and of *Gelidiella acerosa* (Patel *et al* 1986; Subbaramaiah and Thomas 1989) by the bottom culture on coral stones have been developed, and methods for their field cultivation are available with the Central Salt and Marine Chemicals Research Institute. Newer cultivation techniques, such as the single rope floating raft technique (SRFT) which have been practised for *Laminaria* on a commercial scale (Tseng 1981a) and for *Gracilaria* (Li Ren-Zhi *et al* 1984) on a small scale, is tried with *G. edulis* at Krusadai Island in the gulf of Mannar. The efficacy of the SRFT and the possible crop yields obtainable by adopting this technique for *G. edulis* field cultivation forms the subject matter of this paper.

2. Materials and methods

2.1 *Floating raft set-up*

In the SRFT, the main structure is a long coir rope (2·5 cm dia., 30 m long), attached to two wooden stakes with two anchor cables made of synthetic fibres, and kept afloat with bamboo/coconut (kernelless) floats. The length of the cable was 4 m,

which was twice the depth of the sea. Each raft is kept afloat by means of 25–30 floats. The cultivation ropes also of the same diameter coir rope and 1 m in length are attached to the floating rope by hanging. A stone is attached to the lower end of the cultivation rope so as to keep it vertical without floating to the surface. Generally 10 fragments of *G. edulis* are inserted in each rope. The distance between two cultivation ropes is 25 cm and the distance between two rafts is about 2 m (figure 1).

2.2 *Experimental field observations*

An experimental site was selected on the southern side of Krusadai Island in the lagoon at a depth of more than 2 m. *G. edulis* collected from the same locality was used as seed material. Planting was done on 20 m of the cultivation ropes on 25 August 1988. Growth in length of plants was recorded at fortnightly intervals, separately for the plants situated at the upper, middle and lower parts of the cultivation ropes. Harvesting was done once in 3–4 months, up to July 1989 by hand picking leaving behind sufficient biomass of plants for further growth. The monthly extension growth rate was calculated by using the formula, EGR = final length-initial length × 31/number of days of growth and is expressed as cm mon^{-1}. The relative growth rate (RGR, dry wt. g^{-1}d^{-1}) for all harvests was calculated as $RGR = \ln \left(^{w}t/^{w}o\right)/(^{t}t - ^{t}o)$ where ^{w}o and ^{w}t are initial and final dry weights and ^{t}o and ^{t}t are initial and final times, respectively.

3. Results and discussion

3.1 *Growth and crop yield characteristics*

Fragments [4 cm long and 12 g (wet) per one cultivation rope] were planted over 20 m of the cultivation rope. Growth curves for the plants from different levels show differential pattern attaining peak length of 13·5, 15 and 24 cm in the lower, middle and upper parts before harvest in 95 days (figure 2). Mean extension growth rate showed decrease from the top to the middle and to the lower level, that is 8·3, 5·0 and 3·8 (cm mon^{-1}) respectively (table 1). Crop yield also exhibited gradual
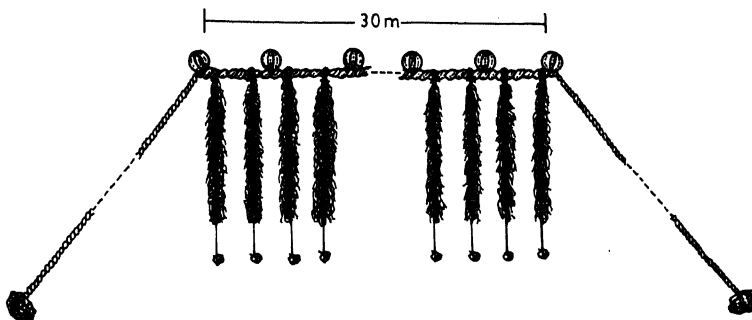
Figure 1. *G. edulis* cultivation raft. Note that each cultivation rope has its upper end tied to the floating rope and its lower end tied to a weighing stone.
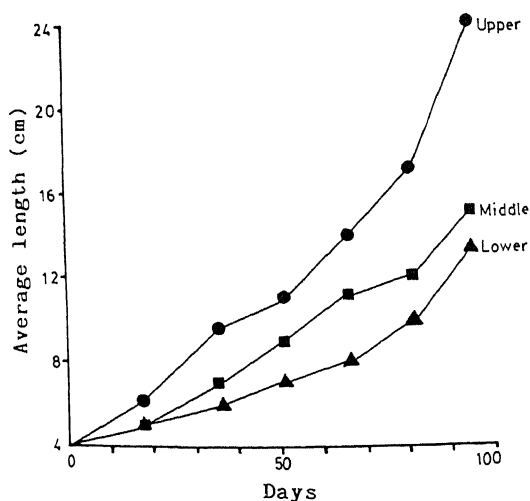
Figure 2. Growth curves (length cm) of *G. edulis* plants grown at the upper, middle and lower parts of the cultivation rope before the first harvest (95 days growth).

Table 1. Growth and harvest data of the experimental cultivation of *G. edulis*.

	Month			
	November	March	July	Total
Days of growth	95	121	96	312
No. of harvest	1	2	3	—
Top				
Extension growth*	7·8	7·4	9·7	—
Crop yield**	2200	1900	1820	5920
Middle				
Extension growth	3·3	5·5	6·3	—
Crop yield	1800	1260	1070	4130
Lower				
Extension growth	2·2	4·4	4·7	—
Crop yield	820	625	630	2075
Mean crop yield**	1606	1262	1173	4041
Relative growth rate (dry wt. $g^{-1} d^{-1}$)	0·05	0·04	0·05	—

*$cm\,mon^{-1}$; **$g\,m^{-1}$ wet.

decrease from top to the lower level (table 1); the plants from the middle and top levels giving 99 and 185% increase respectively over those of the lower level.

The plants grew to maturity in 3–4 months (figure 3) after which a harvest was made, as the plants harvested 3 months after planting were known to yield good quality agar (Thomas and Krishnamurthy 1976). In the 3 harvests made in November 1988, March and July 1989, the biomass crop yield showed gradual decrease, although the relative growth rate remained almost the same 0·04–0·05 g dry wt. $g^{-1}d^{-1}$ (table 1). During growth 98–134-fold increase in biomass was obtained before harvest.

Figure 3. Growth of *G. edulis* plants on the cultivation rope before harvest after 95 days.

In experimental cultivation of *G. edulis* by long line rope method, maximum length of plants up to 30·2 cm and a mean annual crop yield of 3·2 kg (wet) m^{-1} was reported (Raju and Thomas 1971). Later Krishnamurthy *et al* (1977) obtained a mean annual crop yield of 3·43 kg (wet) m^{-1} in scale-up field cultivation of the alga. In the present study, the mean annual crop yield achieved (without reference of the depth) of 4·04 kg (wet) m^{-1} is higher than the yields reported for the alga earlier, and is attributed to the raft technique employed. By the same raft cultivation technique, Li Ren-Zhi *et al* (1984) obtained a much higher yield of 3·3 kg m^{-1} (for *G. verrucosa* and *G. sjoestedtii*) in 5–6 months in the intertidal zone in Qingdao, China. The observed difference in crop yield as compared to that in the present study is probably due to species difference and variations in environmental factors such as temperature, light, nutrients, grazing, etc. The superiority of the raft method lies in growing the plants in the selected water level (Tseng 1981a). In the present study, the variation in the annual crop yield of *G. edulis* from 2·1–5·9 kg (wet) m^{-1} points to a possibility of obtaining greater crop yield at the top level. As maximum yield has been seen in the upper parts, it is suggested that for greater production, the growth on the cultivation ropes can be equalized and maintained at maximum for the alga by tying together the two adjacent hanging ropes of two adjacent rafts, so that the ropes become horizontally disposed instead of hanging vertically.

Acknowledgement

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Speculations on niches occupied by fungi in the sea with relation to bacteria

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Abstract. The significance of fungi in the marine environment is poorly known when compared with the bacteria. Based on information available on bacteria and fungi in the sea and their structural characteristics, the various niches where the latter may play important roles is speculated upon in this article. Among the 3 niches discussed, the fungi, including thraustochytrids may be significant endobionts in substrates such as dead and living plants and calcareous shells. Substantial information is already available on fungi in decaying salt marsh grass and mangrove leaves, seedlings and wood and on parasites in macro- and microalgae. The need to investigate fungi as epibionts on surfaces of various organic and inorganic substrates and also sediments has been stressed. While bacteria are known to be significant as planktonic forms, thraustochytrids and yeasts in this and other niches have not been studied in detail.

Keywords. Fungi; bacteria; sea; niches.

1. Introduction

The role of fungi in terrestrial and freshwater ecosystems is well known when compared with information available on the marine environment. Although fungi occur commonly in the sea as parasites of living organisms and on dead organic matter (Jones 1976; Kohlmeyer and Kohlmeyer 1979; Moss 1986a), their importance in the processes in the sea has still not been sufficiently evaluated. Authors of standard works on marine microbiology and marine ecology (Sieburth 1979; Rheinheimer 1980; Parsons *et al* 1984; Knox 1986) have at most been able to make only vague remarks about their role in the sea. Several papers on the relative roles of bacteria and fungi in the sea have appeared (Fallon and Pfaender 1976; Morrison *et al* 1977; Cundell *et al* 1979; Morrison and White 1980). A few workers have even stated that fungi do not play a major role in the sea (Hanson and Wiebe 1977). These studies, however, have not paid attention to two points. (i) Most marine microbiological studies have not encompassed spatial niches which are likely to harbour great numbers of fungi. One of the objectives of this article is to elaborate that not all niches in the marine environment will be equally conducive to both bacteria and fungi. While the two groups of organisms might share the same niche in certain cases, either one of them might often also dominate certain other niches not preferred by the other. The key to these differences would lie in the structural and physiological characteristics of both these groups (Cooke and Rayner 1984). (ii) The role of yeasts and the unicellular marine microorganisms, the thraustochytrids, which for practical purposes are considered fungi by mycologists, have not been examined.

For the purpose of this discussion, the niches occupied by bacteria and fungi have been divided into 3 categories and each case examined independently.

2. Occupants of various niches

2.1 *Planktonic forms: Utilising nutrients dissolved and uniformly distributed in an aqueous milieu*

Oceanic water contains $0.4–2$ mg l^{-1} dissolved organic carbon (DOC) (Parsons *et al* 1984). Estuarine waters contain considerably more than this (Knox 1986). DOC in seawater arises due to phenomena such as leaching from macrophytes and microalgae, 'sloppy-feeding' of herbivores, autolysis, excretion of animals and terrestrial input (Valiela 1984; Knox 1986; Azam and Cho 1987).

Microorganisms can utilise DOC by living planktonically suspended. A small size is of advantage in this mode of life, by reducing the sinking rate (Hughes 1980) and increasing the surface to volume ratio. Many planktonic bacteria may be as small as 0.2μm in size (Sieburth 1979). The mode of reproduction by bacteria, namely by division into two, followed by separation into two daughter cells results in a rapid dissemination in the aquatic environment. They have very high-affinity transport systems with saturation constants of up to 10^{-8} M for readily assimilable compounds and can efficiently scavenge dilute dissolved organic compounds down to concentrations of a few μg per litre in the water (Pomeroy 1980; Azam and Cho 1987). Bacterial numbers vary from $0.1 \times 10^8 l^{-1}$ in the deep sea to $50 \times 10^8 l^{-1}$ in coastal waters, of which 80–90% may be planktonic (Azam *et al* 1983). Bacteria in the water column of kelp beds can convert up to 29.4% carbon of kelp mucilage into their own cell carbon (Lucas *et al* 1981). Leachates of *Spartina alterniflora* are utilised more efficiently by bacteria than fungi (Fallon and Pfaender 1976). Bacteria were also reported to be more important than fungi in the water column of seagrass beds and sediments (Moriarty *et al* 1985).

There is no proven instance of mycelial fungi growing planktonically in the sea. Although one might isolate a number of fungi from seawater, these either might come from dormant spores or from mycelium in solid debris from terrestrial habitats (Campbell 1983). The larger size of mycelial fungi as compared to bacteria and filamentous habit may not be suited for a planktonic mode of life. On the other hand, the unicellular fungi, yeasts are common in the water column (Fell 1976), although much lower in number than the bacteria. This might be due to the fact that the dissolved organic carbon levels of seawater are too low for the active growth of yeast cells, which are much larger in size than bacteria and have a lower surface to volume ratio and possess saturation constants of only up to 10^{-6} M for easily assimilable compounds (Newell 1984). Where the seawater contains a high amount of dissolved organic carbon, as during algal blooms or in polluted waters, planktonic yeasts may be common (Sieburth 1979). Information on the number and role of yeasts in the sea is meagre and this area needs more detailed investigation.

Among fungi, the unicellular thraustochytrids (Moss 1986b) appear to be the commonest in the sea (Bremer 1976; Miller and Whitney 1981b), with numbers up to 640 cells l^{-1} in the open sea, 6000 cells l^{-1} in coastal waters (Raghukumar and Gaertner 1980) and 10,700 cells l^{-1} in coral reef lagoons (Raghukumar 1987). Vegetative cells of thraustochytrids are $5–20 \mu$m in size. Most known species produce a rhizoid-like ectoplasmic net system which is presumed to aid in penetration and/or attachment to particles and absorption of nutrients (Perkins 1973; Moss 1986b; Coleman and Vestal 1987). The presence of the ectoplasmic net

system would suggest that they live attached to particles. However, at least two species of thraustochytrids, *Althornia crouchii* Jones and Alderman (1971) and *Corallochytrium limacisporum* Raghukumar (1987) do not produce an ectoplasmic net system. Besides, many thraustochytrids display 'mini-cycles' of zoospore-cyst-zoospore which might help these organisms to persist or even thrive planktonically in nutrient poor waters (Kazama *et al* 1975). Still very little is known about their mode of life in the sea and the presently employed cultural techniques (Gaertner 1968; Raghukumar 1986) are not adequate to resolve if thraustochytrids occur as planktonic forms.

2.2 *Endobionts: Organisms utilizing nutrients present within solid substrates*

Dead plants and animals, particularly the former can be utilised by microorganisms either from the surface inwards or nutrients can be directly removed from within as well. Leaves and wood of mangroves, species of seagrasses such as *Thalassia* and *Zostera*, the salt marsh grass *Spartina* and the larger seaweeds such as *Sargassum* and *Laminaria*, as well as filamentous algae all provide such substrates. In coastal waters, macrophytic plants make an enormous contribution to primary production with productivities ranging from 2000–3400 g organic matter/m^2/yr, comparable to that of many cultivated crop plants (Teal 1980; Knox 1986). In the pelagic ecosystem, diatoms and microalgae offer a substrate for microorganisms.

The ability to directly utilise nutrients present within a solid substratum by penetrating and growing within it is one of the most fundamentally important attributes of mycelial fungi (Cooke and Rayner 1984).

The interior of dead plant substrata in the sea may be heavily colonised by fungi. A great amount of work has been particularly carried out on fungi in wood submerged in the sea (Barghoorn and Linder 1944; Kohlmeyer and Kohlmeyer 1979; Mouzouras *et al* 1988). Mycelial fungi are common within dead tissues of the salt marsh grass (Gessner 1977; Newell *et al* 1986), mangrove leaves (May 1975; Fell and Master 1980), mangrove seedlings (Newell 1976) and mangrove wood (Kohlmeyer and Kohlmeyer 1979; Hyde and Jones 1988). Fell and Newell (1980) presented evidence on the role of fungi in carbon and nitrogen immobilization in coastal marine plant litter systems, based on regularly occurring mycoseres, electron microscopy, biochemical indicators and experiments with microecosystems.

Calcareous shells of animals harbour abundant mycelium of microboring fungi within, the fungi probably utilising the organic components in the shells (Kohlmeyer 1969; Rooney and Perkins 1972; Sieburth 1979). Perkins and Halsey (1971) found fungi to be the most widespread and abundant microboring organisms in all their 165 samples of animal calcareous material from continental margin sediments off North and South Carolina, from the intertidal to 758 m depth. The extremely frequent occurrence of fungi in skeletons of reef corals led Bak and Laane (1987) to believe that fungi might play a major role in the coral ecosystem. Foraminiferan tests harbour endobiontic fungi (Kohlmeyer 1984). The importance of mycelial fungi in the recycling of calcareous animal structures in the sea deserves detailed studies.

Fungi have also been reported to be able to penetrate and grow inside a man-made material, polyurethane in the sea (Jones and LeCampion-Alsumard 1970). In general, substrata large enough to support the growth of mycelium and not too

rapidly decomposed by bacteria before the mycelium can establish itself can be expected to harbour filamentous fungi.

Apart from filamentous fungi, the thraustochytrid and labyrinthulid fungi can also penetrate solid substrata. Perkins (1973) demonstrated the penetration of plant cell walls by the ectoplasmic net elements of thraustochytrids and their subsequent growth within the cell lumen. Miller and Jones (1983) have observed thraustochytrids within the kelp, *Fucus serratus* L. Inspite of numerous reports, detailed studies on the role of thraustochytrids in the decomposition of macrophytic plants, and phyto- and zooplankton are not available.

Fungi are important agents of plant diseases on land since they can penetrate and grow within the plant substratum. They could be important plant parasites in the sea as well. Numerous mycelial fungi, mostly belonging to the Ascomycetes parasitise larger thallose algae (Andrews 1976; Kohlmeyer and Kohlmeyer 1979; Porter 1986). Single-celled phycomycetous fungi and thraustochytrids may live as epiparasites on filamentous algae and diatoms. The rhizoids of phycomycetous fungi and the ectoplasmic net elements of thraustochytrids penetrate the host cells and draw nutrients. They may also be endoparasites (Gaertner 1979; Chandralata Raghukumar 1986, 1987; Porter 1986). *Labyrinthula* sp. grow within living tissues of the seagrass *Zostera* and causes the wasting disease (Short *et al* 1987).

Unicellular bacteria are not capable of substantially penetrating solid substrata and growing inside. Even the burrowing bacteria in wood (Mouzouras *et al* 1988) and the actinomycetes have only a limited capability to do so.

This difference in niche between bacteria and fungi has not been sufficiently appreciated by many workers who have looked for mycelial fungi only on the outside of macrophytic material (Morrison *et al* 1977; Bobbie *et al* 1978; Morrison and White 1980). Cundell *et al* (1979) observed fungal infestation of *Rhizophora* leaf surfaces only after 14 days using SEM, whereas Fell and Master (1980) using cultural techniques found them to be present throughout the decomposition of mangrove leaves. Using direct observation as well as other techniques, Newell and Hicks (1982) showed that fungi contributed up to 27% of the volume of decomposing leaf of *Spartina* and bacteria, 0·7%.

In their studies on decomposing salt marsh grass, Benner *et al* (1986) concluded that bacteria were more important than fungi in lignocellulose degradation. However, it would appear that the authors have not observed decompositional stages most likely to harbour lignocellulolytic fungi. Biomass and species of fungi in detritus depend on the stage of decomposition, those actively utilising lignocellulose appearing late (Cooke and Rayner 1984). The biomass of thraustochytrids, likewise may depend on the decompositional stage, such as after leaching out of carbohydrates and phenols (Miller and Jones 1983).

Blum *et al* (1988) concluded that bacterial biomass was considerably higher than that of fungi in seagrass and mangrove detritus. However, the direct microscopy technique involving homogenisation used by these authors might seriously underestimate fungal biomass (Newell *et al* 1986).

In order to assess fungal biomass, the following points will have to be considered: (i) Mycelial fungi should be looked for within solid organic substrata using suitable techniques, (ii) different decompositional stages must be examined and (iii) the presence and abundance of thraustochytrids must be considered.

2.3 *Epibionts: Organisms utilising nutrients present on the surfaces of solid substrata*

The surfaces of both organic and inorganic substrata suspended in an aqueous medium offer a niche for microorganisms. In the former, nutrients could be exuded by the substratum. Larger algae, seagrasses and diatoms constantly exude dissolved organic compounds (Valiela 1984; Knox 1986; Azam and Cho 1987). The external surfaces of living animals may be colonised by microorganisms (Sieburth 1979). The surfaces of animal guts are bathed in nutrients and could provide a niche for bacteria and fungi. In the case of inorganic substrata, various nutrients including biological macromolecules get adsorbed onto the surfaces, thereby offering better conditions for microbial growth than the surrounding water (Campbell 1983; Costlow and Tipper 1984; Characklis and Escher 1988).

Bacteria are the first settlers on surfaces of uncolonised substrata in water. Bacteria growing on the surfaces of dead macrophytes and phytoplankton play an important role in their decomposition (Stuart *et al* 1981; Fukami *et al* 1985; Biddanda and Pomeroy 1988; Blum *et al* 1988). An abundant bacterial flora is found in the guts and on surfaces of animals (Sieburth 1979). On fresh inorganic surfaces, bacteria grow rapidly to form a primary film and are responsible for biofouling and corrosion in the sea (Costlow and Tipper 1984; Characklis and Escher 1988).

Dead macrophytic substrata in the sea internally colonised by fungi may also harbour fungal mycelia on their surfaces (Cundell *et al* 1979; Morrison *et al* 1977; Bobbie *et al* 1978). Fungi grow on surfaces of mangrove prop roots and are avidly fed upon by snails (Kohlmeyer and Bebout 1986). Fungal hyphae may be present on live algal surfaces, although in low numbers (Miller and Whitney 1981a). Fungal hyphae which had penetrated polyurethane coatings were also found on their surface (Jones and LeCampion-Alsumard 1970). Filamentous fungi may not be rare in the guts of marine animals. Hibbits (1978) found large numbers of fungi belonging to Trichomycetes in the guts of 17 of 45 species of Crustacea she examined.

However, mycelial fungi appear to be rare in the primary film of inorganic substrates when compared to bacteria, as judged by the numerous observations using SEM (Sieburth 1975; Bobbie *et al* 1978). This is probably due to the fact that bacteria are at a greater competitive advantage on such surfaces and take up the dilute nutrients more rapidly. However, a particular situation in this connection seems to have been overlooked. The arenicolous or sand-inhabiting fungi (Kohlmeyer and Kohlmeyer 1979; Koch and Jones 1984) draw nutrients from a discrete organic base in the sediment and produce a profusion of hyphae which spread out and grow on the surfaces of sand grains producing ascocarps strongly adhering to them. This space-invading mode of life is one of the characteristics of mycelial fungi and is well known among fungi in terrestrial soils (Campbell 1983; Cooke and Rayner 1984). Many terrestrial species of fungi have also been isolated from marine sediments (Kohlmeyer and Kohlmeyer 1979) and probably live similar to the arenicolous fungi. However, the conventional techniques employed in such isolations do not provide evidence as to whether terrestrial species of fungi grow actively in the marine muds or occur only as dormant spores. Mycelial fungi are highly adaptible and therefore, theoretically it is not impossible for fungi to thrive in aerobic marine sediments.

Yeasts occur on the surfaces of animals, their guts and on seaweeds (Sieburth 1979). A very high number of them might occur during certain stages of decomposition of plant organic matter as has been reported for the salt marsh grass *Spartina* by Meyers (1974). However, little information on their role in surface films of organic and inorganic substrata is available.

Thraustochytrids are common as epibionts on living macrophytes (Sparrow 1969; Miller and Jones 1983) and diatoms (Gaertner 1979; Chandralata Raghukumar 1986). A high number of thraustochytrids are present in the guts of various animals such as sea urchins (Wagner-Merner *et al* 1980), sponges (Höhnk and Ulken 1979; Richter 1985) and hydroids (Raghukumar 1988). These fungi have been isolated from plastic surfaces submerged in the sea (Sparrow 1969). Sediments harbour a high number of 73,000 thraustochytrids per litre (Raghukumar and Gaertner 1980; Jacobsen 1983; Riemann and Schrage 1983). One might expect thraustochytrids to be present on particles as small as 20 μm diameter, since the individual cells of known species of thraustochytrids are 5–20 μm in size. The role of these organisms on various surfaces in the sea deserves attention.

3. Conclusion

It has been rightly pointed out by Karl (1982) that 'future studies in microbiological oceanography and marine biogeochemistry would undoubtedly benefit from a careful analysis of the structure of individual microbial assemblages'. It has been the aim of this article to speculate upon the niches in the sea where fungi might contribute significantly to the microbial assemblage. The utilisation of low concentrations of nutrients in the water column of the oceans might be carried out more efficiently by the bacteria by virtue of their small size and high affinity transport systems. However, the role of fungi in the sea might be greater than hitherto recognized, since niches suitable for growth of different groups of fungi are plentiful as schematically indicated in figure 1. There is strong evidence for an important role for fungi in many niches. (i) Large macrophytic tissue such as that of *Spartina*, mangroves and wood may be colonised extensively by mycelial fungi.
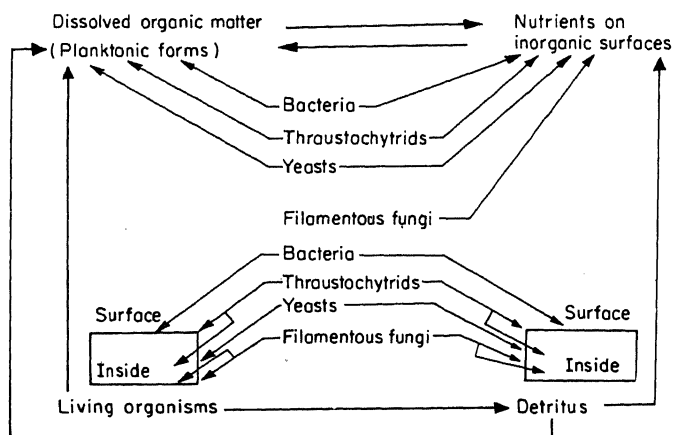
Figure 1. Various niches in the sea occupied by bacteria and different groups of fungi.

(ii) Thraustochytrids and yeasts are present in numbers of up to several hundreds each per litre water in the sea. (iii) Thraustochytrids are extremely abundant in the marine sediments. Thraustochytrids, yeasts and Trichomycetes are common in guts of animals. Numerous situations in the sea have not been adequately explored for fungi. Thus, the role of mycelial fungi in marine muds has still not been satisfactorily answered. The role of thraustochytrids in the decomposition of macrophytic plants as well as phyto- and zooplanktonic organisms has not been investigated. Studies on the role of fungi should consider mycelial fungi, yeasts and thraustochytrids together. A major handicap in resolving many problems on the role of fungi in the sea has been the lack of suitable techniques to estimate the biomass of fungi. Mycelial fungi cannot be estimated in terms of numbers as can be done for bacteria. Their sheltered habitat inside plant substrates adds to the problem. Yeasts and thraustochytrids cannot be easily recognised by microscopic observation of natural samples. Wider application of recent techniques including direct microscopy (Newell and Hicks 1982), use of chemical indicators such as ergosterol (Lee *et al* 1980), fatty acid profiles (Findlay *et al* 1986) and immunological techniques (Newell *et al* 1986; Raghukumar 1988), besides development of newer techniques to estimate fungal biomass might lead to a more complete understanding of the role of fungi and microbial processes in the sea.

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Litter production and seasonality in tropical moist forest ecosystems of Uttara Kannada district, Karnataka

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Abstract. Microlitterfall, ground litter and large wood litterfall were quantified in differently managed forest ecosystems of Uttara Kannada district (lat. 13°55′ to 15°31′N; long. 74°9′ to 75°10′E) with an annual rainfall of 2500 mm largely restricted to 5 months from June–October. Total microlitter produced ranged from 5–10 t/ha/year, ground litter produced ranged from 12–21 t/ha/year and largewood litterfall values ranged from 0·15–1·24 t/ha/year. In all the forest sites leaflitter was found to be major component which constituted 65–92% of the total microlitterfall and 75–94% of the total groundlitter produced. There appeared to be little seasonality in the total microlitterfall in the forest sites with a greater diversity of species, but the seasonal variation was distinct in monoculture and few species dominated vegetation types. Microlitterfall and ground litter production were highest in the dry season and they were negatively correlated with mean monthly rainfall.

Keywords. Leaf litter; litter production; seasonality; tropical forest.

1. Introduction

Litter production is an important pathway for transfer of organic matter and chemical elements from vegetation to soil. It is also an important component of primary production and there have been several studies of litter production in tropical forests including on the Indian subcontinent (Singh and Ramakrishnan 1982; Proctor et al 1983; Prasad and Sharatchandra 1984; Whitmore 1984; Rai and Proctor 1986). The present communication reports for the first time on the levels of litterfall in the moist tropical forests of the western ghats district of Uttara Kannada as a part of an ongoing comprehensive study of the forest ecology of this region.

2. Materials and methods

2.1 *Study area, geology, climate and vegetation*

The studies were carried out in Sirsi and Kumta taluks of Uttara Kannada district (lat. 13°55′ to 15°31′N; long. 74°9′ to 75°10′E) of Karnataka state in peninsular India (figure 1). Geologically this is a transitional zone between the younger basaltic rocks of deccan trap formation and the older crystalline rocks of Archean shield of Indian peninsula. The study sites receive an average rainfall of 2500–3700 mm in Sirsi and Kumta respectively from the south-west monsoon beginning in June and ending in October, with a pronounced dry season from December–April (figures 2, 3). The mean annual maximum temperature in Sirsi ranges from 25–32°C while for
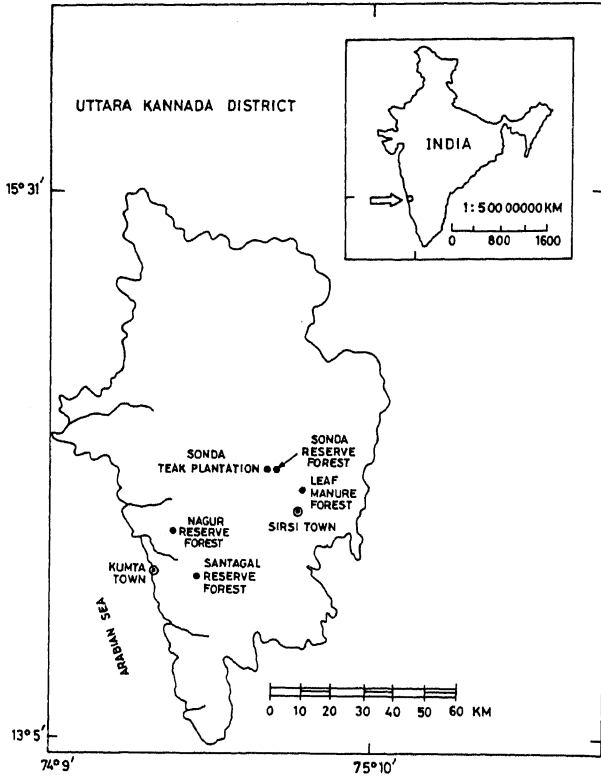
Figure 1. Map of the study area with site locations.
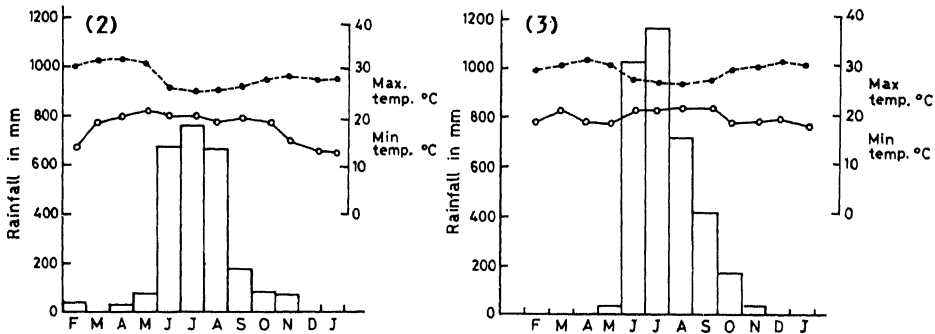
Figures 2 and 3. 2. Climate of Sirsi taluk. 3. Climate of Kumta taluk.

Kumta it is 28–33°C. Mean annual minimum temperature in Sirsi ranges from 13–21°C and in Kumta it is 20–25°C. The vegetation of these localities ranges from dry deciduous to semievergreen with varied degree of biotic disturbances. Five sites were selected to represent leaf manure forest (LMF), reserve forest and teak plantation (table 1).

Table 1. Sampling localities and some physical and other characteristics of the study sites in Uttara Kannda district.

	Leaf manure forest	Sonda reserve forest	Sonda teak plantation	Santgal reserve forest	Nagur reserve forest
Name of the site	Leaf manure forest	Sonda reserve forest	Sonda teak plantation	Santgal reserve forest	Nagur reserve forest
Locality	Bhairumbe, Sirsi	Sonda, Sirsi	Sonda, Sirsi	Santgal, Kumta	Nagur, Kumta
Forest category	Leaf manure forest (= Soppina betta)	Reserve forest	Teak plantation	Reserve forest	Reserve forest
Elevation (MSL)	475 m	475 m	475 m	350 m	105 m
Soil character	Loam soil	Clay loam soil	Clay loam soil	Exposed laterite soil	Exposed laterite soil
Slope	Moderate	Undulating terrain	Gentle slope	Steep slope	Undulating terrain
Management	Assigned to the holder of areca garden under Betta Previlege Act	State Forest Department	State Forest Department	State Forest Department	State Forest Department
Level of biotic disturbance	High	Moderate	Moderate	Little	Moderate
Species richness* (no. of species/ha)					
Tree layer	30	51	12	63	58
Shrub layer	63	61	40	39	63

Table 1. (Contd.)

Population density* (individuals/ha)					
Tree layer	432	692	1,132	964	1,619
Shrub layer	13,480	35,440	5,570	11,490	44,460
Percentage composition					
Evergreen species	43·83	44·77	31·57	86·76	66·66
Desiduous species	56·17	55·23	68·43	13·24	33·34
Floristic composition* Overwood species	Terminalia paniculata, Terminalia alata, Xylia xylocarpa, Pterocarpus marsupium	Terminalia paniculata, Terminalia alata, Xylia xylocarpa, Olea dioica, Dillenia pentagyna	Tectona grandis, Haldina cordifolia, Alstonia scholaris, Ervatamia heyneana	Persea macarantha, Nephelium longana, Diospyros candoleana, Biscofia javanica, Strombosia zeylanica, Pterospermum sp., Holigarna grahmii	Hopea wightiana, Olea dioica, Holigarna arnottiana, Persea macarantha, Pterospermum sp.
Underwood species	Aporosa lindleyana, Careya arborea, Phyllanthus emblica, Buchanania lanzan	Aporosa lindleyana, Flacourtia montana, Ervatamia heyneana, Erhetia sp., Carrisa carandas, Grewia sp., Psychotria sp.	Aporosa lindleyana, Clerodendrum sp., Leea sp., Chromolema odoratum	Nothopodytes foetida, Canthium sp., Nothopegia colebrookiana, Tarena sp.	Aporosa lindleyana, Knema attenuata, Ixora bracheata, Bridelia sp., Psychotria flavida, Glycosmis pentaphylla
Standing biomass** including root (t/ha dry wt.)	Not available	210·10	Not available	259·62	314·35

Source: *Bhat et al (1984); **Prasad et al (1987).

2.2 *Sampling methods*

To retain microlitterfall, i.e., leaf, reproductive parts, small wood ($\leqslant 2$ cm diameter) and trash, and to allow water to drain off an interwoven plastic mesh with small openings (4 mm) was fixed to the mouth of a tetrapod stand to form a 'trap' with a 0.5×0.5 m collecting surface (Proctor 1983; Whitmore 1984) and it was supported at a height of 0.3 m above the ground. Twenty such permanent traps were distributed randomly over an area of one hectare. To collect the ground litter (i.e., microlitterfall + dead herbs) 20 randomly distributed 'quadrats' of 0.5×0.5 m were permanently marked on the forest floor. All the 'traps' and 'quadrats' were cleared initially and the collections were then made once in a fortnight. The collected materials were immediately sorted into (i) leaves, (ii) smallwood ($\leqslant 2$ cm diameter), (iii) reproductive parts viz., flowers and fruits and (iv) trash (demorphosed flowers, fruits, budscales and all other unclassified plant parts). While collecting the groundlitter, the trash was ignored as it was found difficult to distinguish and isolate it from the soil materials.

To collect the largewood litterfall (wood between 2–10 cm diameter), 10 randomly selected quadrats of 10×10 m were permanently marked on the forest floor and all the largewood materials were cleared initially. The collections were made at 4-monthly intervals, i.e., season-wise.

All the collected materials were oven dried to constant weight; the values expressed in the tables are in dry weight. Data from different months were pooled into following 3 seasons:

   I. Summer season (February–May).
  II. Monsoon season (June–September).
III. Winter season (October–January).

The observations were made for two years in case of LMF and for the other sites it was for a period of one year. Statistical analysis was carried out using the method of Zar (1984).

3. Results

3.1 *Litter production*

Estimated values of total microlitterfall ranged from 5 t/ha/year as in Sonda teak plantation to 10 t/ha/year as in Santgal reserve forest (table 2). Of the total microlitterfall, leaf litter constituted 74%, reproductive parts 8% (flowers 2%, fruits 6%), small wood 16% and trash 0.99%.

Estimated values of total ground litter produced ranged from 10 t/ha/year as in LMF to 21 t/ha/year as in Sonda reserve forest (table 3). The groundlitter comprised 83% leaves, 2% reproductive parts (0.30% flowers and 1.70% fruits) and 14% smallwood. Largewood litterfall estimated values ranged from 0.15–1.24 t/ha/ year with an average value of 0.62 t/ha/year (table 4).

3.2 *Seasonality*

While the peak in microlitterfall varied from October to March in the forest sites of

Table 2. Estimated total microlitterfall (t/ha/year) and seasonal variation (total ± SE) on different forest sites in Uttara Kannada district.

Forest site	Season	Leaf	Reproductive parts		Small wood	Trash	Total	Season wise percentage
			Flower	Fruit				
Leaf manure forest*	Summer (February–May)	2·66±0·85	0·0661±0·01	0·1604±0·10	0·273±0·12	0·0375±0·04	3·1996±1·03	47·19
	Rainy (June–September)	0·52±0·07	0·0104±0·008	0·0316±0·008	0·3420±0·11	0·0682±0·03	0·9722±0·15	14·34
	Winter (October–January)	1·99±0·32	0·0252±0·02	0·0721±0·02	0·4900±0·36	0·244±0·009	2·6075±0·43	38·46
	Total	5·17±1·54	0·1017±0·04	0·2641±0·09	1·1050±0·15	0·1299±0·03	6·78±1·62	
Sonda reserve forest	Summer	4·16±1·78	0·23±0·09	1·08±0·70	0·38±0·18	Nil	5·85±2·32	59·81
	Rainy	0·49±0·13	0·02±0·002	0·13±0·01	0·91±0·34	0·08±0·04	1·63±0·45	16·66
	Winter	1·84±0·51	0·13±0·03	0·16±0·05	0·16±0·03	0·008±0·003	2·298±0·56	23·49
	Total	6·49±2·62	0·38±0·14	1·37±0·76	1·45±0·54	0·088±0·06	9·78±3·20	
Sonda teak plantation	Summer	0·9018±0·68	0·001±0·001	0·0136±0·009	0·0235±0·02	0·002±0·001	0·9419±0·71	18·50
	Rainy	0·7655±0·31	0·0034±0·002	0·0270±0·01	0·2954±0·13	0·0169±0·009	1·1082±0·24	21·77
	Winter	3·0192±0·59	Nil	Nil	0·0241±0·01	Nil	3·0433±1·59	59·78
	Total	4·6865±1·78	0·0044±0·002	0·0406±0·01	0·343±0·22	0·0189±0·01	5·09±1·64	
Santgal reserve forest	Summer	2·8642±1·5	0·1206±0·11	0·2344±0·15	0·2683±0·15	0·0222±0·01	3·5052±1·80	34·23
	Rainy	2·0190±0·28	0·0039±0·001	0·0211±0·008	1·4191±0·40	0·0434±0·02	3·5065±0·64	34·24
	Winter	2·4055±0·40	0·1721±0·07	0·0572±0·02	0·5928±0·17	0·0017±0·001	3·2293±0·56	31·53
	Total	7·2887±0·59	0·2966±0·12	0·3127±0·16	2·2757±0·84	0·0673±0·06	10·24±0·22	
Nagur reserve forest	Summer	2·5493±1·11	0·0278±0·02	0·1682±0·03	0·13313±0·08	0·0318±0·02	2·9087±1·17	35·08
	Rainy	1·2804±0·07	0·0017±0·001	0·0662±0·03	0·6195±0·22	0·0600±0·03	2·0281±0·29	24·46
	Winter	2·5647±0·39	0·1321±0·03	0·0057±0·003	0·6345±0·16	0·0154±0·009	3·3526±0·59	40·44
	Total	6·3944±1·04	0·1616±0·09	0·2401±0·03	1·3853±0·03	0·1072±0·13	8·29±0·95	
	Average value	6·06	0·18	0·53	1·30	0·08	8·04	

*Values are average of two years.

Table 3. Estimated total groundlitter values (t/ha/year) and seasonal variation (total ± SE) on different forest sites in Uttara Kannada district.

Forest site	Season	Litter type				Total	Season wise percentage
		Leaf	Reproductive parts		Small wood		
			Flower	Fruit			
Leaf manure forest*	Summer (February–May)	4·47±1·39	0·014±0·01	0·09±0·01	0·63±0·30	5·205±1·27	50·16
	Rainy (June–September)	0·975±0·22	Nil	0·026±0·01	0·385±0·13	1·39±0·26	13·39
	Winter (October–January)	3·205±0·32	0·0122±0·002	0·069±0·01	0·49±0·16	3·78±0·41	36·43
	Total	8·65±2·93	0·0262±0·01	0·185±0·01	1·505±0·12	10·37±2·98	
Sonda reserve forest	Summer	9·58±2·37	0·08±0·05	0·83±0·11	0·39±0·17	10·89±2·79	51·22
	Rainy	3·57±0·88	Nil	0·036±0·02	1·03±0·35	4·64±1·14	21·82
	Winter	4·90±1·14	0·04±0·01	0·05±0·01	0·72±0·13	5·71±1·27	26·95
	Total	18·05±4·44	0·12±0·05	0·916±0·64	2·14±0·45	21·14±4·71	
Sonda teak plantation	Summer	2·43±1·09	Nil	0·02±0·02	0·03±0·03	2·48±1·09	20·47
	Rainy	2·30±1·00	0·0001±0·0009	0·017±0·009	0·46±0·20	2·77±1·10	22·87
	Winter	6·79±0·88	0·0001±0·0001	0·003±0·003	0·06±0·04	6·86±0·87	56·64
	Total	11·52±3·61	0·0011±0·001	0·04±0·01	0·55±0·33	12·11±3·45	
Santgal reserve forest	Summer	5·95±1·90	0·05±0·03	0·10±0·10	0·96±0·42	7·06±2·40	36·84
	Rainy	3·76±0·29	0·0007±0·0004	0·006±0·001	2·32±0·38	6·08±0·62	31·73
	Winter	4·70±0·68	0·05±0·03	0·03±0·02	1·24±0·17	6·02±0·73	31·42
	Total	14·41±1·54	0·1007±0·04	0·136±0·07	4·52±1·01	19·16±0·82	
Nagur reserve forest	Summer	5·83±1·76	0·002±0·003	0·068±0·05	0·718±0·09	6·61±1·59	41·92
	Rainy	2·82±0·34	0·0002±0·0001	0·032±0·01	1·11±0·22	3·86±0·47	24·50
	Winter	4·27±0·45	0·007±0·005	1·017±0·0009	1·004±0·19	5·29±0·62	33·58
	Total	12·92±2·12	0·0092±0·005	0·117±0·03	2·733±0·23	15·77±1·94	

*Values are average of two years.

Table 4. Estimated large wood litterfall (t/ha/year) and seasonal variation on different forest sites in Uttara Kannada district.

| Forest site | Period of observation | Season | | | Total |
		Summer (Feb.–May)	Rainy (June–Sept.)	Winter (Oct.–Jan.)	
Leaf manure forest*	1–2–1985 to 26–1–1987	0·2385	0·2219	0·2574	0·71 ± 0·02
Sonda reserve forest	5–11–1985 to 31–10–1986	0·1700	0·0700	0·1700	0·41 ± 0·08
Sonda teak plantation	19–11–1985 to 31–10–1986	0·0530	0·0410	0·0640	0·15 ± 0·01
Santgal reserve forest	27–12–1985 to 15–12–1986	0·1400	0·4700	0·6500	1·24 ± 0·36
Nagur reserve forest	27–12–1986 to 15–12–1986	0·1400	0·0700	0·4000	0·61 ± 0·24
Average value		0·1483 ± 0·026	0·1745 ± 0·071	0·3082 ± 0·091	0·62 ± 0·16

*Values are average of two years.

Sirsi taluk (figures 4–6), microlitterfall was more or less uniform in the forest sites of Kumta taluk (figures 7, 8). If all the forest sites are considered together, the largewood litterfall showed significant difference among seasons (Friedman test $X^2_{(3, 0.05)} = 7.30$) (table 4) while the difference among seasons was not significant for total microlitterfall ($X^2_{(3, 0.05)} = 1.20$) (table 2) and total ground litter produced ($X^2_{(3, 0.05)} = 3.60$) (table 3). However, the difference among the seasons for leaf litter component was found significant in microlitterfall ($X^2_{(3, 0.05)} = 7.6$) and in ground litter produced ($X^2_{(3, 0.05)} = 8.6$). Highest total microlitterfall (77%) and leaf litterfall (83%) was observed in dry period and lowest in the wet period. Within a forest site, the seasonal variation was remarkable with respect to total microlitterfall, total groundlitter produced and also their major components (see tables 2, 3).

While the monthly production of total microlitter, groundlitter and leaf litter in all the forest sites showed negative correlations with mean monthly rainfall, the small wood of microlitterfall showed positive correlation. The monthly total microlitterfall in Sonda teak plantation showed a strong negative correlation ($r = -0.74$) with mean monthly temperature, but in Nagur reserve forest it showed positive correlation ($r = 0.55$) and in the remaining forest sites it showed a weak negative correlation. Table 5 summarises the testing for seasonal variations for total microlitterfall, ground litter produced, leaflitterfall and smallwood litterfall on different forest sites and table 6 shows the correlation with rainfall and temperature.

4. Discussion

4.1 *Litter production*

In spite of large difference between the forest sites in floristic composition, species
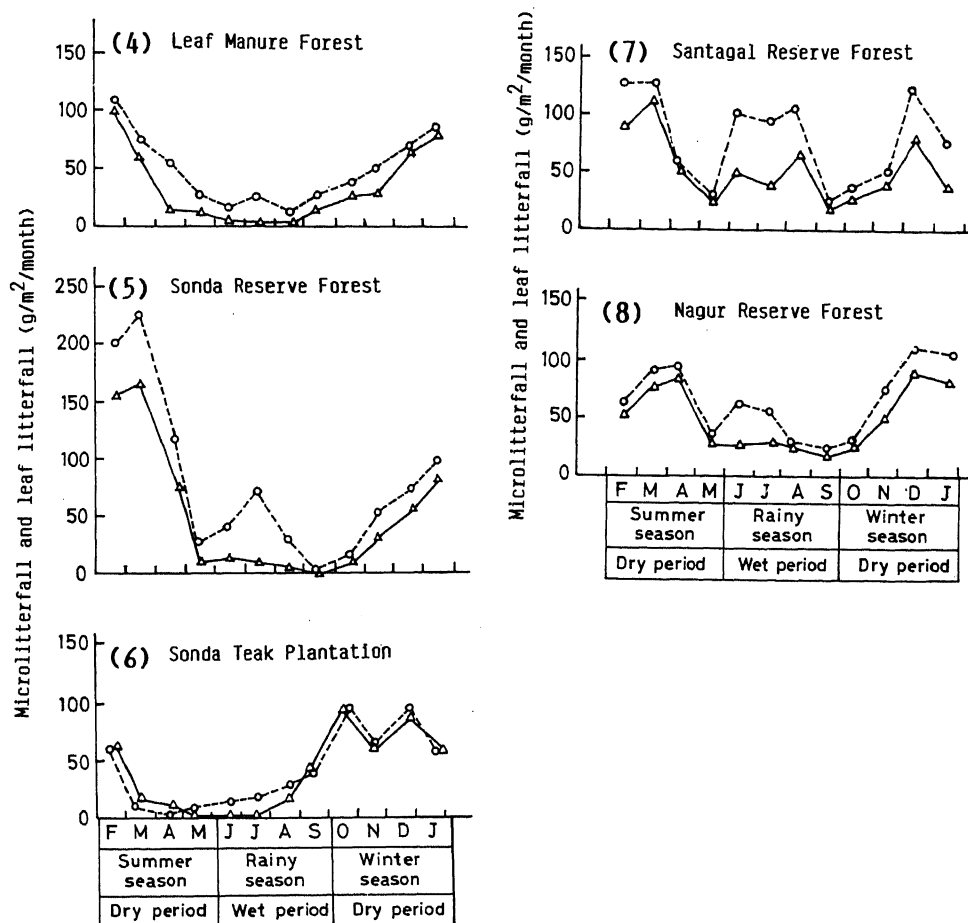
Figures 4–8. Seasonality in microlitterfall (○) and leaf litter fall (△) on various forest sites of Uttara Kannada district.

richness, population density, level of biotic disturbance, age and standing biomass, the total microlitterfall values ranged 5–10 t/ha/year with an average of 8·04 t/ha/year which is well within the range of other estimated values (table 7). The measurements of annual litterfall and annual leaffall are often used to estimate the net primary productivity of a forest and the annual leaffall is considered to represent one-third of NPP (Bray and Gorham 1964). If that is the case, the estimate of net primary productivity for Sonda teak plantation is 14·07 t/ha/year, for LMF is 15·51 t/ha/year, for Nagur reserve forest is 19·17 t/ha/year, for Sonda reserve forest is 19·87 t/ha/year. As these values are within the range of other estimated values, the present estimates of litterfall and leaffall appear to be reasonable.

The wide range (12–21 t/ha/year) in the estimated values of ground litter production may be because of herblayer production which is mainly dependent upon abiotic and biotic factors (Bhat and Gadgil 1987), higher density of individuals and canopy structure (Prasad *et al* 1987) and also because of inclusion

Table 5. Testing for seasonal variation for total microlitterfall, total ground litter, leaf litter and small wood litter components on various forest sites in Uttara Kannada district.

Forest sites		Total microlitterfall			Leaflitter component			Smallwood component microlitterfall			Total groundlitter produced			Leaflitter component of groundlitter			Smallwood component of groundlitter		
		Summer Vs Rainy	Summer Vs Winter	Rainy Vs Winter	Summer Vs Rainy	Summer Vs Winter	Rainy Vs Winter	Summer Vs Rainy	Summer Vs Winter	Rainy Vs Winter	Summer Vs Rainy	Summer Vs Winter	Rainy Vs Winter	Summer Vs Rainy	Summer Vs Winter	Rainy Vs Winter	Summer Vs Rainy	Summer Vs Winter	Rainy Vs Winter
Leaf manure forest		+++	+	+	+++	+	++	+	+++	+	+++	+++	+++	+++	++	+++	+	+	+
	df	12	12	14	12	12	14	12	12	14	12	12	14	12	12	14	12	12	14
	t	3·65	1·63	0·96	3·42	1·76	2·14	0·14	3·37	2·09	5·49	3·38	4·20	4·5	2·89	4·80	0·82	0·27	0·76
Sonda reserve forest		++	++	+	++	+	+++	+	+	+	+++	+++	+	++	+	+	+	+	+
	df	12	12	14	12	12	14	12	12	14	12	12	14	12	12	14	12	12	14
	t	3·03	2·80	0·94	2·90	2·04	16·09	0·66	0·63	2·08	3·87	3·86	0·61	2·41	2·18	0·97	1·08	0·84	0·77
Sonda teak plantation		+	++	++	+	++	++	+	+	+	+	++	+++	+	+++	+++	+	+	+
	df	11	11	12	11	11	12	11	11	12	10	10	10	8	10	12	10	10	12
	t	0·01	2·49	3·45	0·30	2·28	2·5	1·91	0·12	2·16	0·53	2·81	3·51	0·90	4·32	3·80	2·10	0·43	2·16
Santgal reserve forest		+	+	+	+	+	+	+	+	+	++	+	+	+	+	++	+	+	+
	df	11	10	13	11	10	13	11	10	13	11	10	13	11	10	13	11	10	13
	t	1·3	1·70	0·81	1·86	1·06	1·40	1·75	1·06	1·50	2·58	2·02	0·008	0·83	2·06	2·23	0·21	0·27	1·90
Nagur reserve forest		++	+	+++	++	+	+	+	++	+	+++	++	++	+++	++	++	+	+	+
	df	11	10	13	11	10	13	11	10	13	11	10	13	11	10	13	11	10	13
	t	2·69	0·71	3·09	2·91	1·01	2·16	1·31	2·32	0·39	4·65	2·84	2·82	4·21	2·57	2·17	0·43	0·005	0·43

+, No significant difference; + +, significant difference at 5% level; + + +, significant difference at 1% level.
df, Degree of freedom.

Table 6. Correlation coefficient of various litter types and their major components with rainfall and temperature on various forest sites in Uttara Kannada district.

	Forest sites				
	Leaf manure forest	Sonda reserve forest	Sonda teak plantation	Santgal reserve forest	Nagur reserve forest
Rainfall					
Total microlitterfall	−0·84	−0·54	−0·04	−0·03	−0·31
Total ground litter	−0·56	−0·72	−0·37	−0·69	−0·69
Leaflitter of microlitterfall	−0·80	−0·74	−0·40	−0·50	−0·51
Leaflitter of ground litter	−0·62	−0·63	−0·37	−0·85	−0·36
Small wood of microlitterfall	0·40	0·52	0·62	0·38	0·56
Small wood of ground litter	0·20	−0·11	0·68	0·35	0·19
Large wood litterfall	−0·50	−1·00	−0·50	0·50	−0·50
Temperature					
Total microlitter fall	−0·02	−0·09	−0·74	−0·06	0·55
Total ground litter	0·05	0·28	−0·59	0·10	0·54
Leaflitter of microlitterfall	−0·35	0·02	−0·62	−0·07	0·46
Leaflitter of ground litter	0·06	0·31	−0·62	0·06	0·43
Small wood of microlitterfall	0·38	−0·10	−0·08	0·02	0·44
Small wood of ground litter	−0·21	−0·51	−0·02	0·05	0·40
Large wood litterfall	−0·50	0·50	−0·50	0·50	0·50

Table 7. Total microlitterfall (t/ha/year) in a range of tropical forests.

Author(s)	Forest type and locality	Production (t/ha/year)
Bhat (present communication)	Tropical moist forest ecosystem Uttara Kannada district, Karnataka (present study)	5·09–10·24
Garg and Vyas (1975)	Deciduous forest, Udaipur, Rajastan	6·00
Gong and Ong (1983)	Coastal hill dipterocarpus forest pantai, Acheh Forest Reserve, Penang Island	7·45
Leigh and Windsor (1982)	Tropical forests, Barro Colarado, Panama	10·00 (7·5 leaves and 2·5 wood)
Proctor *et al* (1983)	Low land rain forest, Gunung Mulu National Park, Sarawak	8·8–12·0
Rai (1981)	Tropical rain forest, Western Ghats of Karnataka	3·44–4·18
Rai and Proctor (1986)	Tropical rain forests of Western Ghats of Karnataka	3·44–4·20
Rodin and Bazilevich (1967)	Tropical rain forests, Yunan, China	11·6
Schaik (1986)	Rainforest, Sumatra	9·59–13·22
Singh and Ramakrishnan (1982)	Sub-tropical forest, Meghalaya	5·5
Songwe *et al* (1988)	Tropical rainforest, Southern Baakundu forest reserve, Cameroon	12·9–14·1

of dead herbaceous litter. Largewood litterfall values which ranged from 0·15–1·24 t/ha/year are similar to other reported values (Edwards 1977). The variation in annual litter production is attributed to species diversity (Barbour *et al* 1980), latitude (Bray and Gorham 1964), successional stages (Singh and Ramakrishnan

1982) and insect herbivory (Franklin 1970). It appears that the litterfall is higher on the sites like Sonda reserve forest, Santgal reserve forest and Nagur reserve forest which have a larger tree population, greater mix of species and minimum biotic disturbance (see table 1). However, the lower rate of litterfall observed in Sonda teak plantation is mainly because of its age coupled with biotic disturbance, while for the LMF it is mainly because of periodic harvest of green matter by the user of the LMF which curtails the transfer of plant parts to soil from the vegetation.

4.2 *Seasonality*

Wet and dry seasons have strong influence on the community structure and leaf growth, flowering, fruiting and shedding of parts seem to be influenced by alternating wet and dry seasons (Smith 1980). It appears that maximum litter production and leaf fall occurred in the dry period and minimum in wet period. However, within a forest site the seasonal variation is remarkable for total microlitterfall, total ground litter produced and also for other litter components. If all the forest sites considered together the difference among seasons is not significant for total microlitterfall ($X^2 = 1.2$) and total ground litter produced ($X^2 = 3.6$). But for the leaf litter component the difference among seasons is significant ($X^2 = 7.6$ and $X^2 = 8.6$). This indicates that though leaflitter dominated in both total microlitterfall and total ground litter, it alone is not determining the seasonal patterns of total microlitterfall and total ground litter production.

Studies in tropical regions report peak litter production and leaffall coinciding with either low precipitation (Hopkins 1966; Daubenmire 1972; Gong and Ong 1983; Lam and Dudgeon 1985; Songwe *et al* 1988) or high precipitation (Conforth 1970; Edwards 1977; Brassel *et al* 1980). Litterfall and leaffall are often attributed to photoperiod (Alvim and Alvim 1978), drought (Medina 1983), insect damage (Colley 1982; Leigh and Windsor 1982; Dirze, 1984), rise in temperature (Schaik 1986), rainfall (Proctor *et al* 1983) and high velocity winds (John 1973). From the present study it appears that the peak litter production and leaffall is during dry period and it varied from October to March, during which the rainfall is minimum. This is supported by the observed negative correlation of total microlitterfall and leaf litterfall with mean monthly rainfall (cf. table 6). However, smallwood of micro-litterfall showed positive correlation with rainfall. This clearly indicates that leaf shedding is markedly seasonal as it is a species specific phenomenon (Kunkel–Westphal and Kunkel 1979; Rai and Proctor 1986; Songwe *et al* 1988) and wood litterfall is largely governed by physiological processes (Christensen 1975). Drying of tender shoots might have occurred during dry summer and the observed positive correlation of smallwood litterfall with rainfall may be the result of high velocity monsoon winds and mechanical stress due to gain in weight by imbibing rain water.

Wider spread in peak microlitterfall (i.e., from October to March) is mainly because of floristically diverse forests and also due to interspecific differences in leaf shedding time of deciduous and evergreen species. This is clearly shown in synchronised shedding of leaves in monoculture such as Sonda teak plantation with a peak in October which showed strong negative correlation ($r = -0.74$) with temperature. Similarly, LMF which is also like monoculture, as few selected species are maintained by the user, showed a peak in February and negative correlation with temperature. But a stand like Nagur reserve forest which has a greater

diversity of species showed positive correlation with temperature indicating a varied response of species to temperature. Total lack of seasonality as observed in Santgal reserve forest is probably due to constant input of individual litter component by different species year-round.

5. Conclusion

Litter production is dependent upon the level of disturbance, species richness, population density, standing biomass and possibly the age of the stand. Though the seasonality is not remarkable in the total microlitterfall and the total ground litter produced, it is more pronounced in leaf litter component and also in large wood litterfall. Highest leaf litter production is in the dry period and such remarkable seasonality in leaf production is related to the floristic composition of the stand.

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Nutrient acquisition by fungi—the relation between physiological understanding and ecological reality

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Abstract. The measure of the ability of a fungus to acquire a soluble nutrient is given by the flux of that nutrient across the plasma membrane. The determination of fluxes into fungi, the factors which govern the magnitude of a flux, particularly external substrate concentration, and pH are discussed. The difficulties of determining fluxes in the natural environment are considered. Reference is made to studies on unicells, bacteria and fungi, in enhancing understanding of the relationship between growth and the flux of a nutrient into a fungus. The artificial system is considered to be a valuable step between laboratory experiment and ecological reality. Likewise, the generation of mathematical models relating flux of nutrient to growth can provide an insight as to the significance of various physiological processes influencing growth of a fungus in its natural environment.

Keywords. Membrane flux; natural environment; growth limitation; artificial systems; theoretical models.

1. Introduction

The acquisition of nutrients by a fungus is dependent on their transport across the plasma membrane of those hyphae involved in exploiting a substrate. An important part of the acquisition process might also be the breakdown of substrate molecules which are insoluble or the wrong molecular size for entry into a hypha.

A great deal is known now about the mechanisms of transport of solutes into particular fungi, principally *Neurospora crassa* and *Saccharomyces cerevisiae* (Borst-Pauwels 1981; Cooper 1982; Eddy 1980, 1982; Sanders 1988; Slayman 1987). There is also significant information for *Aspergillus*, *Penicillium* and *Schizosaccharomyces* (Goffeau and Boutry 1986; Jennings 1976a). A decade ago, it might have been dangerous to speculate how far our knowledge of membrane transport processes obtained from this taxonomically rather narrow range of fungi could apply across the whole fungal kingdom. There is now sufficient evidence to indicate that in general terms membrane transport processes are very similar in all fungi, based on a proton economy as has been established for *N. crassa* and *S. cerevisiae*.

Evidence exists that a similar economy exists in a range of species across the fungal kingdom, i.e., *Achlya bisexualis* (Kropf 1986), *Dendryphiella salina* (Davies et al 1990), *Metschnikowia reukaufii* (Gläser and Höfer 1987), *Phanerochaete chrysosporium* (Greene and Gould 1984), *Phytophthora megasperma* (Giannini et al 1988), *Thraustochytrium aureum* (A Garrill and D H Jennings, unpublished results). The ubiquity of the proton economy in fungal membrane transport means that external pH has important consequences for nutrient acquisition.

Though there is considerable knowledge about the molecular basis of membrane transport in particular fungi which is likely to be applicable to a range of others,

there has been little work on the effectiveness of particular transport systems in the growth of a fungus under natural conditions. By effectiveness, I mean the ability of the transport system to supply the metabolic machinery of the mycelium with nutrients, sufficient at least for the requirements for maintaining the protoplasmic fabric but more properly to allow growth and reproduction. Here I discuss how we might proceed in bridging the gap between physiological knowledge and ecological reality. Inevitably, what I have to say is short on particulars and long on generalities. Nevertheless, I hope the burden of the text will be of a kind to encourage a more concerted attempt to bridge the gap.

While certain solutes may diffuse into fungal hyphae, e.g. undissociated buyric acid into *N. crassa*, virtually all nutrients acquired by fungi cross the plasma membrane via protein carriers. Indeed, that such carriers are part of a system bringing about accumulation of the solute within the mycelium means that the system can act in a scavenging role, which will be very important in those habitats where nutrients may be in low concentration. If nutrients were to move into hyphae by diffusion, scavenging the medium of nutrients could only be effectively carried out by a metabolic process in the cytoplasm immediately converting the entering nutrient into another compound incapable of exit back into the medium. Only in this manner it would be possible to generate at the inner face of the plasma membrane a sink for diffusion inwards close to zero concentration.

2. Membrane fluxes

The ability of a fungus to acquire a nutrient at any one concentration and under prescribed environmental conditions is given by the flux of that nutrient across the plasma membrane, namely the number of mol passing across unit area of it (cm^{-2}) per unit time (s^{-1}). Values for the flux of a nutrient into particular fungi can be used to assess the competitive ability for a particular nutrient. Clearly that fungus with the higher flux rate will accumulate more of the nutrient. Thus the higher flux of phosphorus into beech mycorrhizal roots compared to those which are non-mycorrhizal is believed to underpin the better growth of mycorrhizal seedlings of beech compared with those which are non-mycorrhizal (Harley and McCready 1950).

Even though there is this striking example there are few flux data of any kind for fungi. This is because virtually all studies are in terms of dry matter rather than surface area. Even those values which have been obtained are for the most part only assessments, because the surface area has not been measured directly but calculated from fresh and dry weights and mean hyphal diameters (Jennings and Aynsley 1971; Slayman and Slayman 1968). There is a need for flux values calculated on the basis of the determined surface area.

The determination of the surface area of the plasma membrane through which the flux might be occurring is now relatively simple with stereology an established discipline (Clipson *et al* 1989). Essentially, mycelium has to be examined by transmission electron microscopy for the appropriate values to be obtained. This is not the place to consider the criteria which must be met to obtain statistically acceptable values for the surface area of the plasma membrane; the reader should consult other authors, e.g. Weibel (1979). Here it is more important to stress that physiologically acceptable values for plasma membrane fluxes demand the use of

young mycelium. If we are considering liquid culture, with increasing pellet size there will be increasing uncertainty (i) about the physiological state of the plasma membrane throughout the mycelium and (ii) the extent to which the concentration of solute in the medium is anywhere near uniform external to the plasma membrane. As the pellet becomes larger, declining oxygen tension within the pellet will affect directly the rate of transport but also other metabolic requirements, while a non-uniform concentration will confound the determination of nutrient flux (number of mol entering the mycelium) (Trinci 1970).

Whatever the age of the mycelium, there is the question as to the extent to which nutrient transport systems are distributed uniformly along hyphae or if distributed uniformly are operating at the same rate throughout the mycelium. We know for instance that proton extrusion via the plasma membrane ATPase only becomes active some distance from the hyphal apex (Galpin and Jennings 1975). Equally, as a hypha grows older the transport rate could decline due to feedback inhibition consequent upon the accumulation of a particular nutrient metabolic product of that nutrient as is the case with methionine and sulphate transport (Jennings 1976a). There may be decline consequent upon increased passive leakage driven by the increased diffusion gradient between inside and outside. By alluding to these possibilities, it is clear that mycelium cannot be considered necessarily as a well-stirred system, such that cytoplasmic concentrations along a hypha are uniform. Non-uniformity of monovalent cation concentrations along the apical compartment of a hypha have been demonstrated (Galpin *et al* 1978).

I have been speaking about uptake of a nutrient in terms of its flux across the plasma membrane without reference to the dependence of that flux on the external concentration of the nutrient. The relationship between flux (J) and concentration at its simplest is given by

$$J = \frac{J_{max} \cdot S}{S + k},\tag{1}$$

where J_{max} is the maximum flux possible, S the external solute concentration and k the affinity constant of the transport system. The value of k is clearly important if we wish to determine how the flux changes with external nutrient concentration. However the above relationship holds only for a transport system with a single binding site which behaves irreversibly, i.e. brings about a totally unidirectional flux of nutrient across the membrane. We now know that the kinetics of transport are much more complex due to the fact that solute movement across the plasma membrane is driven by the proton electrochemical potential gradient. Thus the binding of a proton to the carrier can affect the affinity of the solute for the carrier and the rate of movement across the membrane of the carrier with or without the solute, such that the kinetics for transport are complex (Sanders 1986). However, if we are thinking about ecological situations, where there might be low nutrient concentrations, then, at constant pH, the kinetics of transport will increasingly approximate to the simple relationship given above.

3. Fluxes in the natural environment

It should be clear from the foregoing that, if we are to understand in terms of nutrient uptake how a fungus grows in its natural environment, we need to know

the concentration of nutrients and the pH at the locale where the fungus is growing. There is no doubt about the challenge of obtaining this information for ideally it means direct determination not extrapolation from bulk measurements. Nevertheless, studies on soil have shown how the availability of nutrients in a complex substratum may be assessed (Nye and Tinker 1977). However there are now possible approaches to the direct determination of local nutrient concentrations which ought to be explored.

For instance, if one can obtain a sample of the substrate in which the fungus is growing (and that also presupposes that one can measure the rate of growth of that fungus within its substrate) one could examine the sample and determine by X-ray microanalysis at least the elemental composition of the environment immediately around a hypha and obtain a close approximation to the concentration of particular elements. While there are no problems about potassium, for other elements such as phosphorus one will have to make assumptions as to the chemical form in which the element is found. On the other hand, it may be possible to subject a similar piece to nuclear magnetic resonance (NMR) spectroscopy in which case not only might it be possible to determine what phosphate compounds are present but also, if there is sufficient orthophosphate present to give a signal, it will be possible to determine the pH within the substratum. Local pH might also be determined using pH micro-electrodes or microscopically using a dye the degree of fluorescence of which is pH dependent. We must have more information about the pH in the immediate vicinity of fungi growing in their natural environment, particularly since the fungus itself may alter the pH. The problem of buffering liquid media and the rapidity at which the pH may change in unbuffered media, as a result of fungal growth, highlights this situation. However, it is not axiomatic that the pH will change. Under certain conditions a fungus can grow exponentially without the pH of the medium being changed (Borrow *et al* 1964). Further, any pH change will be dependent upon the buffering capacity of the environment. Finally, it needs to be remembered that a change in pH will not only affect the rate of nutrient acquisition directly but may influence it indirectly by changing the availability (and hence external concentration) of a nutrient. An excellent example of this is the increased availability of phosphate to higher plants as a result of the acidification of the soil by a fungus (Khan and Bhatnagar 1977; Kucey 1987).

I have considered here how one might approach the problem of assessing the ability of a filamentous fungus to acquire nutrients in its natural environment. The procedures described ought to produce data which might be similar to that obtained in the laboratory. It might be possible to obtain data to evaluate the competitive ability for a nutrient under natural conditions. However, it is clear that to obtain meaningful information is beset by technical difficulties. Thus I have minimised the fact that any values for the concentration of a nutrient in the locale of a fungus will be for only a point in time. The maintenance of any particular concentration will depend on the rate of supply either by release from insoluble material or by bulk flow of solution or diffusion of the solute to the hypha from other parts of the local environment. There is also the matter of translocation of nutrients within hyphae as a means of supplying nutrients to the growing hyphal tips from another part of the habitat. Fortunately, it is now becoming possible to assess rates of movement of nutrients in the medium external to hyphae and translocation inside them in micro-habitats such that the values obtained and

analysis of the processes involved are relevant to situations such as in soil (S Olsson and D H Jennings, unpublished results).

Many of the problems of trying to relate rates of nutrient acquisition by mycelial fungi in their natural habitats with values from laboratory studies appear close to being intractable. Of course, when one considers those fungi with macroscopic organs many of the technical problems are of a lesser order of magnitude. It has already been demonstrated by Harley and McCready (1952) with beech mycorrhizal roots and Clipson *et al* (1987) with cords of *Mutinus caninus* that it is possible to obtain important information from studies in the field. Further in the case of studies on nutrient acquisition by cords it is possible to use microcosms in the laboratory. In the nutrient economy of cords and rhizomorphs, translocation is a key process but again there are feasible procedures for assessing how the process might move nutrients from one part of the cord to the other. However, for cords and rhizomorphs it is not necessarily so much a matter of relating laboratory studies, i.e. studies in totally artificial systems such as those used by Granlund *et al* (1985) and Jennings (1990), to studies in the field or in microcosms but using the variety of procedures available to answer specific questions. One such question is the effectiveness of adventitious hyphae arising from rhizomorphs and probably also cords in the absorption of nutrients (Cairney *et al* 1988).

4. Growth limitation

There is a need to consider the extent to which the rate of acquisition of nutrients might limit growth under natural conditions. Here work on bacteria provides useful guidance. Neijssel and Tempest (1976) showed that, when *Klebsiella aerogenes* was grown in glucose limited chemostat culture, at all growth rates up to values close to the maximum the sudden addition of glucose to such a culture led to an immediate stimulation of respiration rate. Subsequently, the same was subsequently shown to be the case for *Escherichia coli* (Neijssel *et al* 1977). If growth were limited by transport capacity, then addition of glucose would not result in an increase in oxygen consumption rate. Indeed Neijssel *et al* (1977) showed after extra glucose was fed to *K. aerogenes* products of glucose metabolism, gluconate, pyruvate and acetate were found in the medium indicating that the rate limiting steps are located after the transport step possibly at the pyruvate dehydrogenase or in the pentose phosphate pathway. I have argued from less precise information that the net flux of a nutrient required to support the growth of a filamentous fungi is much less than the maximum which can be achieved by manipulating the physiological state of non-growing mycelium (Jennings 1976b).

Neijssel *et al* (1977) have argued that, when a mixed population of microorganisms is growing in a nutrient limited environment, only those species which can withstand the severe competition for the limiting nutrient will be successful. These authors pointed out that microorganisms generally possess high affinity transport systems for all potentially limiting nutrients. Furthermore, there is now very good documentation for the ability of microorganisms to increase their uptake affinity for a nutrient when it becomes limiting (Harder and Dijkhuizen 1983; Poindexter 1987). On the other hand, if a particular nutrient limitation is suddenly relieved those organisms which can express a higher uptake rate will take that nutrient up and express a faster growth rate. In support of this argument,

Neijssel *et al* (1987) indicated that what had been observed for glucose as described above had also been observed for potassium, magnesium, phosphate and sulphate, namely when one of these nutrients is added to cultures limited with that particular nutrient oxygen consumption is stimulated.

The presence of high affinity transport systems in fungi for many nutrients has been well authenticated. It should be noted that for any one nutrient such a system has not often been demonstrated in many fungi. But there is no reason to argue that the ideas of Neijssel *et al* (1977) are not applicable to fungi. However, as I have argued elsewhere (Jennings 1987), in natural environments it may not be the supply of combined carbon which may be limiting but metals. Nevertheless, whatever the limiting nutrient, the fluxes of the other nutrients may be greater than what is required to support growth *per se*. These fluxes may be responsible for allowing the fungus to store nutrients such that it is better able to exhibit sustained growth when the supply of the limiting nutrient is removed.

The reference to the importance of metals to the growth and functioning of fungi should remind us that certain species, under conditions when there is low concentration of iron in the medium, produce ferric-iron-specific ligands (siderophores) which bind iron and take it into the protoplasm (Winkelmann 1986). In such instances the rate of growth of the fungus might be limited as much by the flux of the ligand out of the hyphae as by the flux of ligand plus metal into the hyphae.

Thus far, I have been assuming that the investigator has knowledge about the properties of the membrane transport processes operating in a fungus and is attempting to use that knowledge to interpret rates of growth or indeed the presence in (in contrast to the absence of other species) a particular habitat. It will be clear that much depends on the determination of the local concentration of nutrient(s) under consideration and there are distinct difficulties in making such determinations.

5. Artificial systems

The other approach and one advocated by myself at an earlier date (Jennings 1987) is to use model or artificial systems which mimic important features of the habitat in which a fungus might be found. It is not appropriate here to go into detail about the procedures which can be used; a number were referred to in the former article (Jennings 1987). In terms of what is being discussed here, any procedure used must be such as to allow the investigator to have some control over the concentration of nutrients available to the fungus or fungi being studied. Of the procedures available, the chemostat is probably the simplest system in environmental terms, though, because the environment is kept constant, in technical terms the system is complex. Nevertheless it is appropriate to finish by reference to a chemostat study which has been used to explain a simple ecological situation but which also demanded knowledge of the transport processes within the fungal species involved.

The study is that by Postma *et al* (1989) who were concerned with competition between the yeasts *S. cerevisiae* and *Candida utilis* for glucose in glucose-limited chemostat culture. Under aerobic conditions *C. utilis* always dominated over *S. cerevisiae*. Under anaerobic conditions however the reverse occurred. It is believed that under aerobic conditions the high affinity glucose/proton symport of

C. utilis competes much more successfully for the hexose in solution than the relatively low-affinity facilitated diffusion system for glucose in the cells of *S. cerevisiae*. This provides a molecular explanation of the fact that when *S. cerevisiae* is grown commercially to produce baker's yeast the process can become contaminated with wild yeasts such as *Candida* (Fowell 1967). Since the process involves batch cultivation of *S. cerevisiae* under aerobic conditions and sugar limitation, one can see the results of the chemostat study are immediately relevant. The need now is to identify examples from the natural environment which can be probed in a similar manner by the use of an artificial system whose specifications have been judiciously chosen to relate to the habitat under consideration.

6. Theoretical models

This matter of nutrient limitation for a fungus within its natural environment can be considered from another standpoint. Growth of a microorganism can be described mathematically (Pirt 1975). However there have been few attempts to relate growth to the flux of nutrients into a microorganism. One can see that if a suitable mathematical relationship can be devised then it might be possible to use it to elucidate what might be governing growth of a fungus in its natural habitat. Thus, if it were possible to determine both the concentration of a nutrient deemed to be limiting growth of the fungus within its habitat and the rate of growth of the fungus, use of the relationship might demonstrate whether or not the flux of the nutrient into the mycelium of the fungus were limiting its growth.

I have produced such a relationship for the growth of a filamentous fungus (Jennings 1976b). The relationship describes growth in the presence of a constant concentration of the limiting nutrient. The final equation derived was

$$J = \frac{r}{2} \cdot \frac{dC}{dt} + \frac{C}{l} \cdot \frac{L_{max} \cdot C}{C + K_m}, \tag{2}$$

where J is the flux of nutrient into the fungus, r and l the radius and the length of the hyphae respectively, C the internal concentration of the nutrient, L_{max} the maximum rate of growth (increase in length) and K_m is the Michaelis constant for an enzyme reaction involving the nutrient which has a controlling effect on growth. Application of the equation depends on knowledge of dC/dt the rate at which the concentration of nutrient in the mycelium changes. Dealing with this term might be intractable were it not for the fact that in certain instances—and this seems true for potassium and glucose—the problem is removed by the term being zero. In the case of potassium, particularly for marine fungi growing in the presence of relatively high concentrations of sodium chloride, the concentration remains constant over a wider range of growth rates (Jennings 1976b; Burke and Jennings 1990). On the other hand, in the case of glucose metabolism in many members of the Ascomycotina the situation is such that the hexose on entering the fungus is usually immediately converted into other metabolites particularly polyols (Jennings 1984).

The other matter, concerns the choice of a value for K_m. In the case of potassium this is not easy because this cation is metabolically inert but acts as a cofactor for a large number of enzymes (Evans and Sorger 1966). Nevertheless, it has been possible to show by judicious choice of a K_m for potassium that for reasonable rates

of growth of the fungus *D. salina* there needs to be only relatively low fluxes of the ion into the mycelium ($0.13–0.47$ p mol cm^{-2} s^{-1}) (Jennings 1976b). The relationship between growth and the flux of glucose has not been investigated in the manner outlined above but, for this metabolite, if one were to study the growth of an ascomycetous species, the choice of K_m would be most likely to either that for hexokinase or that for mannitol dehydrogenase (NADP$^+$) (Jennings 1984).

In the case of marine fungi or other fungi growing in saline media, the nutrient or solute absorbed may be significant not because it might be involved in metabolism or be converted into the structure of the cell but because it might contribute to the osmotic potential of the mycelium such that sufficient turgor is generated for growth. When ascomycetous or related fungi are growing in saline conditions with a sufficiency of glucose, glycerol is a major component of the internal osmoticum (Adler and Gustafsson 1980; Beever and Burns 1986; Brown 1978; Burke and Jennings 1990; Wethered *et al* 1985). When *Debaryomyces hansenii* is grown under glucose limitation in alkaline (pH 8·3) conditions or under potassium limitation, sodium (probably as chloride) makes the major contribution to the internal osmotic potential (Burke and Jennings 1990). Thus, for fungi growing in saline conditions, which can be considered also as being conditions of lowered water availability, i.e. low water activity or low water potential (Griffin 1981; Jennings 1990), we need to know the extent to which growth is primarily limited by the generations of turgor. Hence the concern will be about the magnitude of the fluxes of those solutes making the major contribution to the osmotic potential and hence to turgor. Thus, for a fungus growing in saline conditions, there is a need to consider not only the solute movements between the external medium and the fungus but also its water relations. This is exemplified most clearly by the fundamental relationship for the volume change of a walled-cell as a function of the osmotic driving forces (Dainty 1963)

$$dV/dt = A L_p (\sigma\pi - P), \tag{3}$$

where V is the volume of the cell, t is time, A the surface area of the cell, L_p the hydraulic conductivity of the outer membrane, σ the reflection coefficient of the same membrane, π the osmotic gradient across the membrane and P the turgor pressure within the cell.

As one can see from eq. (3) there are important determinants of water uptake, two which are properties of the outer membrane, namely the hydraulic conductivity and the reflection coefficient and the other a function of the ability of the cell to do osmotic work, namely the osmotic gradient. The generation of the osmotic gradient depends upon the effectiveness of transport processes in the outer membrane. The reflection coefficient is a measure of that osmotic effectiveness because it is a measure of the semi-permeability of the membrane. A completely semi-permeable membrane has a value of unity; decreasing degrees of semi-permeability will lead to values of less than unity. Loss of solutes from the cell will tend to drive the reflection coefficient below unity.

When considering fungi, it seems that for the most part one can consider the hypha or the cell in osmotic terms as a single compartment system. The evidence available, though limited, indicates that vacuoles play only a minor osmotic role (Clipson *et al* 1989; Clipson and Jennings, 1990). Therefore when considering the osmotic properties of a fungus it seems likely that it is only the properties of the plasma membrane which need to be considered. That said, we have almost no

information about the hydraulic conductivity or the reflection coefficient of the plasma membrane of fungi. There is some indication that the hydraulic conductivity might not be very different from that found for green plant cells (D H Jennings, unpublished results). On the other hand, there are some indications that fungi might behave under certain circumstances as if they had a membrane with a reflection coefficient of less than unity. Here one is referring to the frequent observation of loss of glycerol from halotolerant fungi in the presence of increasing or decreasing saline conditions (Brown 1978; Adler *et al* 1985; André *et al* 1988), i.e. the influx of combined carbon (glucose) by the fungus can be accompanied by an increased efflux of combined carbon (glycerol) such that the membrane appears much more permeable to combined carbon.

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Degradation of α-, β- and γ-isomers of hexachlorocyclohexane by rhizosphere soil suspension from sugarcane

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Abstract. A suspension of soil from the rhizosphere of sugarcane plants grown from sugarcane setts treated with a commercial formulation of hexachlorocyclohexane effected exceptionally rapid degradation of α- and γ-isomers of hexachlorocyclohexane in a mineral salts medium under aerobic conditions. β-Hexachlorocyclohexane was also degraded, but slowly. No degradation of γ-hexachlorocyclohexane occurred in the medium inoculated with sterilized sugarcane rhizosphere soil suspension. During degradation, about 40% of the ^{14}C from the ring-^{14}C-γ-hexachlorocyclohexane was released as CO_2 indicating ring cleavage. A bacterium, *Pseudomonas* sp., isolated from hexachlorocyclohexane-treated sugarcane rhizosphere soil readily degraded γ-hexachlorocyclohexane added to a mineral salts medium as a sole source of carbon under aerobic conditions. An increase in temperature from 20–25 and 30°C progressively increased the degradation of α- and γ-hexachlorocyclohexane by rhizosphere soil.

Keywords. Hexachlorocyclohexane isomers; sugarcane rhizosphere; aerobic degradation; temperature effect.

1. Introduction

Insecticides constitute more than 75% of all pesticides used in India and probably many other countries in the tropics and subtropics (Mrinalini 1983). A broadspectrum organochlorine insecticide, hexachlorocyclohexane (HCH) alone accounts for more than 56% of all pesticides used in India (Anonymous 1984). HCH is used especially for controlling insect pests of important crops such as rice and sugarcane. It is a common practice to treat sugarcane setts with HCH before planting for protection against termites and other common pests of sugarcane. Soil application of HCH significantly increased the yield of sugarcane besides exhibiting insecticidal properties (Singh and Sandhu 1964).

Commercial formulations of HCH generally contain α, β, γ and other isomers of which γ-isomer is the most insecticidal and β-isomer the most persistent. HCH isomers persist in aerobic soil and water systems, but undergo very rapid degradation in predominantly anaerobic flooded soil and other anaerobic ecosystems (Raghu and MacRae 1966; Sethunathan *et al* 1983). Recently, however, aerobic biomineralization of α-HCH in a soil slurry from a heavily HCH-contaminated site has been reported (Bachmann *et al* 1988a,b). Sugarcane rhizosphere is known to harbour large populations of microorganisms capable of degrading an organochlorine herbicide, 2,4-dichlorophenoxyacetic acid (Loos 1975; Sandmann and Loos 1984). The relative ability of the rhizosphere soil from sugarcane plants, previously treated with a commercial formulation of HCH, to degrade α-, β- and γ- isomers of HCH under aerobic conditions was studied.

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2. Materials and methods

2.1 *Preparation of rhizosphere soil suspension*

Sugarcane setts were treated with a commercial formulation of HCH and then planted in soil. Sugarcane plants were carefully removed from the soil and the roots gently tapped to remove the large soil particles and clods. The root system with closely adhering soil (rhizosphere) was then shaken with 100 ml of sterile distilled water for 1 h and the resulting suspension was used as sugarcane rhizosphere soil suspension.

For non-rhizosphere soil suspension, 2 g of soil collected from an unplanted plot (adjacent to the sugarcane plot) was shaken with 100 ml of sterile distilled water.

2.2 *Degradation of HCH isomers*

A mineral salts medium [$(NH_4)_2HPO_4$, 0·5 g; $MgSO_4.7 H_2O$, 0·2 g; $FeSO_4.7H_2O$, 0·001 g; K_2HPO_4, 0·1 g; $Ca(NO_3)_2$, 0·01 g and distilled water, 1 litre, pH 7] was equilibrated with technical grade γ-HCH (purity 99·1%) for 24 h and then sterilized by filtration through a Millipore filter (0·3 μm). Ten ml portions of this medium were dispensed in sterile 100 ml Erlenmeyer flasks and then inoculated with 0·1 ml of the rhizosphere soil suspension from sugarcane or non-rhizosphere soil suspension. Uninoculated medium served as control. The uninoculated and inoculated media were incubated under aerobic conditions by shaking at room temperature ($26\pm3°C$). At periodic intervals, 1 to 2 ml aliquots of inoculated and uninoculated media from each of the duplicate flasks were withdrawn aseptically, shaken with 1–5 ml of hexane and 50 mg of sodium sulphate for 2 min and analysed for γ-HCH by gas-liquid chromatography (GLC).

In another experiment, α-, β- and γ-HCH were dissolved in acetone and 0·5 ml of acetone containing 50 μg of the respective isomer was added to separate sterile 100 ml Erlenmeyer flasks. After evaporation of acetone at room temperature for about 12 h, 10 ml aliquots of sterilized mineral salts medium were added to each flask, shaken for 24 h and then inoculated with 0·1 ml of sugarcane rhizosphere soil suspension. At every sampling, after incubation at room temperature, two flasks each of uninoculated and inoculated media for each isomer were shaken with 10–20 ml of hexane for 20 min and HCH isomers in hexane fraction were analysed by GLC.

To study the effect of temperature, α- and γ-HCH were equilibrated with the mineral salts medium for 48 h and 10 ml portions of this medium, sterilized by passing through a Millipore filter (0·3 μm), were added to sterile 100 ml flasks. The medium was incubated under aerobic conditions, at 20, 25, 30 and 35°C in BOD incubators. At regular intervals, 1 to 2 ml of the medium from each of the duplicate flasks were analysed for the respective HCH isomer.

2.3 *Evidence for biodegradation*

A 25 ml portion of sugarcane rhizosphere soil suspension was sterilized by autoclaving at 121°C for 30 min. Ten ml portions of the medium containing γ-HCH

in aqueous solution were inoculated separately with 0·1 ml of sterilized and nonsterilized sugarcane rhizosphere soil suspension and then incubated at room temperature under aerobic conditions for 6 days. At periodic intervals, 1 to 2 ml of the medium from duplicate samples were analysed for γ-HCH.

Ring cleavage of aromatic molecules leading to CO_2 evolution is mediated essentially by microorganisms. For further evidence for microbial role in the degradation of γ-HCH, a suspension of sugarcane rhizosphere soil was tested for its ability to mineralize ^{14}C-γ-HCH. Ten ml portions of the mineral salts medium, supplemented with ^{14}C-γ-HCH (1×10^5 dpm/ml), were dispensed into 100 ml Erlenmeyer flasks and then inoculated with 0·1 ml of rhizosphere soil suspension. Each flask containing uninoculated or inoculated samples was closed with a rubber bung provided with an inlet and an outlet which were closed with a pinchcock. The assembly was incubated at $26 \pm 2°C$ under aerobic conditions. At 5 and 10 days, the inlet was connected to an air generator through a trap containing 25 ml of 2 N KOH solution to remove the $^{14}CO_2$, if any, in the air and $^{14}CO_2$ that evolved from the ^{14}C-γ-HCH in each flask was purged into 10 ml of carbon cocktail (repurged with nitrogen) containing pseudocumene (R J Harvey Instrument Corporation, 123, Patterson Street, New Jersey, USA). The radioactivity remaining in the medium after $^{14}CO_2$ evolution was extracted with 10 ml of hexane and 1 ml aliquots of the hexane fraction were mixed with 5 ml of optiphase Hi-safe 11 liquid scintillation cocktail (flash point 144°C) in a 10 ml scintillation vial (FSA Laboratory Supplies, Loughborough, Leics, UK). Radioactivity in the aqueous phase remaining after hexane extraction was determined by mixing 1 ml aliquots of the aqueous phase after hexane extraction with 10 ml of the same scintillation solution. The radioactivity in different fractions (CO_2, hexane-extractable, aqueous phase) was assayed in a Rackbeta liquid scintillation counter model 1209 (LKB Wallac, Finland) with chemical and colour quenching correction. DPM conversion with background correction was printed on a Facit B 1100 printer. Counting efficiency was 96·3%.

For further confirmation of bacterial role in the aerobic degradation of γ-HCH, mineral salts medium amended with γ-HCH (2 to 3 μg/ml) was inoculated with a suspension of HCH-treated sugarcane rhizosphere soil. When γ-HCH disappeared from the inoculated medium, 5 ml of this medium was inoculated into fresh mineral salts medium supplemented with γ-HCH as the sole source of carbon. After 10 repeated transfers for selective enrichment of HCH-degrading microorganisms, 0·1 ml of the enriched medium was plated on the mineral salts agar medium supplemented with γ-HCH (2 to 3 μg/ml). Four individual bacterial colonies differing in morphological and growth characteristics were tested for their ability to degrade γ-HCH in 10 ml mineral salts medium supplemented with γ-HCH (3 μg/ml) as a sole source of carbon. At periodic intervals, 1 ml aliquots of the samples were withdrawn from duplicate flasks of each isolate, residues of γ-HCH extracted in 1 ml of hexane and analysed by GLC.

2.4 GLC

HCH isomers, extracted by shaking the samples with hexane, were analysed in a gas chromatograph (Perkin-Elmer, model 3920) equipped with a ^{63}Ni detector and a glass column (0·625 cm OD; 2 m length) packed with 5% QF-1 on Chromosorb

W, 60/80 mesh. Column, injector and detector were maintained at 190, 210 and 250°C, respectively with a flow rate of carrier gas (argon) at 60 ml/min. Under these conditions, the retention time was 0·75 min for α-HCH, 1·25 min for β-HCH and 1 min for γ-HCH. The recovery of all the 3 isomers from the medium ranged from 90–95% by this method.

3. Results and discussion

Sugarcane rhizosphere soil suspension effected distinctly more rapid degradation of γ-HCH than the non-rhizosphere soil suspension under aerobic conditions. γ-HCH added in aqueous solution completely disappeared in 5 days after inoculation of the medium with sugarcane rhizosphere soil suspension under aerobic conditions (table 1). In medium inoculated with non-rhizosphere soil suspension about 38% of the γ-HCH was recovered even after 10 days. In uninoculated medium, decrease in its concentration was negligible even after 10 days.

Degradation of α-, β- and γ-HCH by sugarcane rhizosphere soil was studied. α-HCH disappeared almost at the same rate as γ-HCH and both reached low levels in 6 days after inoculation (table 2). In contrast, about 69% of the added β-HCH was recovered from the inoculated medium even after 24 days. Evidently, β-HCH appeared to be more resistant to degradation than α- and γ-isomers. In this experiment, the 3 isomers were added to provide a final concentration of 5 μg/ml of each isomer in the medium. Since the water solubility of α- and β-isomers was less than 5 μg/ml, the residues in the whole flask were extracted and analysed in this experiment. The assumption was that the undissolved portion will come into solution when the isomer in solution is microbially degraded. This assumption appeared to be logical since all the added α-isomer was degraded in 5 days although its water solubility is below 2 μg/ml.

In another study, the rate of degradation of α- and γ-HCH increased with increase in temperature from 20–25°C and from 25–30°C (tables 3 and 4). A further rise in temperature from 30–35°C appeared to slow down their degradation while accelerating the volatilization loss. Both isomers disappeared completely in 6 days after inoculation at 25°C and in 4 days at 30°C. At 20°C, about 50% of the γ-

Table 1. γ-HCH recovered from a mineral salts medium inoculated with rhizosphere soil suspension from sugarcane under aerobic conditions.

Incubation (days)	γ-HCH[a] recovered (μg.ml^{-1} of medium)		
		Inoculated	
	Uninoculated	Rhizosphere	Non-rhizosphere
0	6·0 ± 0·6[b]	6·6 ± 0·4	6·5 ± 0·2
2	6·0 ± 0·2	5·5 ± 0·3	5·4 ± 0·5
5	5·8 ± 0·2	0	2·9 ± 0·1
10	5·5 ± 0·3	0	2·5 ± 0·2

[a] γ-HCH was incorporated to the mineral salts medium in aqueous solution.
[b] Mean of duplicate estimations ± deviation.

Table 2. HCH (α, β and γ) recovered from a mineral salts medium inoculated with sugarcane rhizosphere soil suspension under aerobic conditions.

Incubation (days)	HCH recovered (μg.ml^{-1} of the medium)					
	α-HCH[a]		β-HCH[a]		γ-HCH[a]	
	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated
0	5·0±0·1[b]	5·2±0·1	5·0±0·1	4·8±0·2	5·0±0·2	5·6±0·2
6	3·3±0·3	0·2±0·1	ND	ND	4·1±0·1	0·1±0
12	3·2±0·5	0	4·8±0	4·0±0	4·0±0·1	0
24	ND	ND	4·6±0·1	3·5±0·3	ND	ND

[a]HCH isomers were added to each flask in acetone and after evaporation of acetone, equilibrated with mineral salts medium to provide a final concentration of 5 μg.ml^{-1}.
[b]Mean of duplicate estimations ± deviation.
ND, Not determined.

Table 3. γ-HCH recovered from a mineral salts medium inoculated with sugarcane rhizosphere soil suspension and incubated at different temperatures.

Incubation (days)	γ-HCA[a] recovered (μg.ml^{-1} of the medium)							
	20°C		25°C		30°C		35°C	
	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated
0	6·5±0·1[b]	6·5±0·05	6·4±0·1	6·4±0·1	6·5±0·05	6·6±0·1	6·5±0·1	6·4±0·05
2	6·3±0·05	6·3±0·1	6·0±0·1	5·9±0·1	5·8±0·03	5·2±0·2	4·6±0·2	4·5±0·1
4	5·8±0·08	5·6±0	5·0±0	3·5±0·1	5·0±0·2	0	3·6±0·1	1·5±0·8
6	5·5±0·1	3·2±0·2	5·0±0·1	0	4·2±0	0	3·4±0·1	0

[a]γ-HCH was incorporated to mineral salts medium in aqueous solution.
[b]Mean of duplicate estimations ± deviation.

Table 4. α-HCH recovered from a mineral salts medium inoculated with sugarcane rhizosphere soil suspension and incubated at different temperatures.

Incubation (days)	α-HCH[a] recovered (μg.ml^{-1} of the medium)							
	20°C		25°C		30°C		35°C	
	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated
0	1·7±0[b]	1·7±0	1·7±0	1·7±0	1·7±0	1·7±0	1·7±0	1·7±0
2	1·7±0·03	1·5±0·1	1·4±0·04	1·3±0·01	1·3±0·05	1·2±0·03	0·9±0·03	0·8±0·03
4	1·4±0·02	1·1±0·04	1·1±0·02	0·9±0·1	1·1±0·02	0	0·6±0·01	0
6	1·2±0·05	1·0±0·01	0·8±0	0	0·7±0·03	0	0·5±0·01	0

[a]α-HCH was incorporated to mineral salts medium in aqueous solution.
[b]Mean of duplicate estimations ± deviation.

isomer and 71% of the α-isomer were recovered after 6 days of inoculation. There was some loss of both isomers from uninoculated medium, possibly due to volatilization. However, the disappearance rate of both isomers was always more pronounced in inoculated medium than in uninoculated medium at all temperatures.

The concentration of γ-HCH declined to undetectable levels in medium

inoculated with nonsterilized suspension within 6 days under aerobic conditions (table 5); but in medium inoculated with sterilized suspension, the decrease in the concentration of the insecticide was negligible during the corresponding period. Moreover, until 4 days, the decline in γ-HCH level was not considerable in the medium inoculated with nonsterilized suspension; but the insecticide completely disappeared from the medium between 4 and 6 days. The initial lag and the subsequent rapid loss of γ-HCH only from the medium inoculated with nonsterilized suspension suggest microbial role in its degradation.

Isotope studies showed that radioactivity in the hexane extract of the medium declined to less than 6% of the original level in 5 days after inoculation of the medium with rhizosphere soil suspension under aerobic conditions with a concomitant, but not proportional, increase in the radioactivity in the aqueous phase (table 6). Interestingly, about 37% of the ^{14}C in γ-HCH was released as $^{14}CO_2$ in 5 days after inoculation and incubation beyond 5 days was not effective in further mineralization. In uninoculated control, decrease in the radioactivity in the hexane phase was negligible after incubation for 10 days and evolution of $^{14}CO_2$ was

Table 5. γ-HCH recovered from a mineral salts medium inoculated with sterilized and nonsterilized rhizosphere soil suspensions from sugarcane.

Incubation (days)	γ-HCH[a] recovered ($\mu g.ml^{-1}$ of the medium)	
	Sterilized	Nonsterilized
0	$7 \cdot 0 \pm 0 \cdot 2$[b]	$6 \cdot 9 \pm 0 \cdot 1$
2	$6 \cdot 7 \pm 0 \cdot 1$	$6 \cdot 3 \pm 0$
3	$6 \cdot 8 \pm 0 \cdot 02$	$6 \cdot 0 \pm 0 \cdot 2$
4	$6 \cdot 7 \pm 0 \cdot 2$	$5 \cdot 3 \pm 0 \cdot 4$
5	$6 \cdot 7 \pm 0 \cdot 1$	$2 \cdot 8 \pm 0 \cdot 4$
6	$6 \cdot 6 \pm 0 \cdot 1$	0

[a]γ-HCH was incorporated to mineral salts medium in aqueous solution.
[b]Mean of duplicate estimations $\pm$ deviation.

Table 6. Distribution of radioactivity during degradation of uniformly ring-^{14}C-γ-HCH by sugarcane rhizosphere soil suspension in a mineral salts medium under aerobic conditions.

	Radioactivity recovered (%) 10 ml^{-1} of medium[a]					
	Uninoculated			Inoculated		
Incubation (days)	Hexane fraction	Aqueous fraction	CO_2	Hexane fraction	Aqueous fraction	CO_2
0	$94 \cdot 7 \pm 0 \cdot 64$[b]	0	0	$98 \cdot 7 \pm 2 \cdot 0$	$0 \cdot 8 \pm 0 \cdot 04$	0
5	$85 \cdot 4 \pm 1 \cdot 54$	$0 \cdot 65 \pm 0 \cdot 1$	$0 \cdot 04 \pm 0 \cdot 01$	$5 \cdot 7 \pm 0 \cdot 8$	$17 \cdot 3 \pm 0 \cdot 57$	$36 \cdot 7 \pm 7 \cdot 5$
10	$73 \cdot 9 \pm 0 \cdot 34$	$0 \cdot 84 \pm 0 \cdot 2$	$0 \cdot 17 \pm 0 \cdot 01$	$4 \cdot 1 \pm 0 \cdot 2$	$12 \cdot 7 \pm 0 \cdot 6$	$38 \cdot 1 \pm 0 \cdot 03$

[a]^{14}C-γ-HCH was added at 1×10^5 dpm 10 ml^{-1} of medium.
[b]Mean of duplicate estimations $\pm$ deviation.

Table 7. γ-HCH[a] recovered from a mineral salts medium inoculated with bacterial cultures isolated from sugarcane rhizosphere.

Incubation (days)	γ-HCH[a] recovered (μg.ml^{-1} of medium)				
	Uninoculated	I_1^b	I_2^b	I_3^b	I_4^b
0	$3\cdot2\pm0\cdot1^c$	$3\cdot1\pm0\cdot2$	$3\cdot2\pm0\cdot3$	$3\cdot0\pm0\cdot1$	$3\cdot0\pm0\cdot2$
1	$3\cdot1\pm0\cdot2$	$0\cdot26\pm0\cdot04$	$1\cdot5\pm0\cdot5$	$2\cdot2\pm0\cdot3$	$2\cdot5\pm0$
2	$3\cdot0\pm0\cdot3$	0	$0\cdot08\pm0\cdot02$	$2\cdot2\pm0\cdot4$	$2\cdot3\pm0\cdot2$
3	$3\cdot1\pm0\cdot1$	0	0	$2\cdot1\pm0\cdot1$	$2\cdot0\pm0\cdot3$

[a]γ-HCH was incorporated to mineral salts medium in aqueous solution.
[b]Bacterial isolates.
[c]Mean of duplicate estimations $\pm$ deviation.

negligible. Substantial ring cleavage of γ-HCH by rhizosphere soil suspension also suggests the participation of microorganisms in its degradation.

HCH isomers are known to be highly volatile. The disappearance of α- and γ-HCH only from the inoculated medium and not from the uninoculated medium suggests that this decrease is due to their degradation and not due to volatilization of the parent molecules. However, no metabolites were detected in the gas chromatograms during aerobic degradation of α- and γ-HCH. There are reports of the formation of metabolites, but in small, and not stoichiometric amounts, during degradation of γ-HCH in microbial cultures, soils and soil suspensions (MacRae *et al* 1969; Brahmaprakash *et al* 1985; Bachmann *et al* 1988a,b). These metabolites (γ-TCCH and γ-PCCH) appear to be more volatile than the parent molecule (Tsukano and Kobayashi 1972) and possibly escape to the environment as volatiles immediately after their formation. Also, Haider and Jagnow (1975) found that during anaerobic degradation of γ-HCH in microbial cultures, a substantial portion of the ^{14}C in ^{14}C- γ-HCH was not accounted for. This was attributed to the formation of chlorine-free volatile metabolites. However according to our study, more than 50% of the ^{14}C in ring-^{14}C- γ-HCH was accounted for in different fractions (CO_2, hexane-extractable and aqueous phase) during aerobic degradation of γ-HCH by sugarcane rhizosphere soil.

Microbial role in the aerobic degradation of γ-HCH by rhizosphere soil from HCH-treated sugarcane was confirmed by isolating HCH-degrading bacteria. All the 4 bacterial isolates (I_1 to I_4) degraded γ-HCH added to the mineral salts medium as a sole source of carbon under aerobic conditions (table 7). The most active isolate I_1 was Gram negative, nonsporeforming, motile, oxidase-positive and catalase-positive and was identified as *Pseudomonas* sp. based on these and other morphological and biochemical characteristics (Buchanan and Gibbons 1974). Further detailed studies on the degradation of other HCH isomers by this bacterium are underway.

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Interactive effect of NaCl salinity and gibberellic acid on shoot growth, content of abscisic acid and gibberellin-like substances and yield of rice (*Oryza sativa* L. var GR-3)

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Abstract. When rice (*Oryza sativa* L. var GR-3) plants were subjected to salt stress (12 dS/m) the extension growth and dry weight of the shoot system as well as the content of chlorophyll and gibberellin-like substances were found to be markedly reduced. Contrarily, the level of abscisic acid in the shoot system registered a rapid and massive increase in response to salinity. Compared to control, salt stressed plants showed higher concentration of Na^+ and Cl^- and lower concentration of K^+ in the leaf tissue. Salinization also resulted in a considerable reduction in grain yield. Exogenous application of gibberellic acid (10 ppm) significantly increased the growth and yield of salt stressed plants. Gibberellic acid treatment reduced the net accumulation of Na^+ and Cl^- and maintained high level of K^+ in the leaves of salinized plants. A significant fall in the content of abscisic acid and a marked increase in the amount of chlorophyll were also noticed in salinized plants in response to gibberellic acid administration. These results suggest that gibberellic acid improved the growth and yield of stressed rice plants presumably by hormonising the ionic status of the plants as well as by modulating the endogenous level of abscisic acid.

Keywords. *Oryza sativa*; abscisic acid; gibberellic acid; growth; salinity.

1. Introduction

Soil salinity is one of the major constraints which limits crop productivity (Abrol 1986). The reduction in plant growth and yield could result from the toxic effects of ions (Na^+ and Cl^-) on metabolism or from adverse water relations (Yeo 1983). A number of studies carried out in the past clearly indicate that hormonal regulation is involved in the control of membrane permeability and water relations (Ilan 1971; Karmoker and Van Steveninck 1979). Mizrahi *et al* (1971) and Boucaud and Ungar (1976) observed a marked decrease in the levels of gibberellins and cytokinins and an abrupt rise in abscisic acid (ABA) content in salt stressed plants and suggested that the salt induced changes in membrane permeability as well as water relations and the subsequent growth reduction can be attributed to the altered endogenous hormone contents. An earlier study in this laboratory showed that gibberellic acid (GA_3) is capable of reducing the toxic effects of NaCl on germination and seedling growth of rice (Acharya 1983). The present paper reports the results of further investigations undertaken to evaluate the potential of GA_3 in mitigating the adverse effects of salt stress on growth and yield of rice.

2. Materials and methods

2.1 *Plant material and growth conditions*

Seeds of *Oryza sativa* L. var GR-3 were obtained from the State Department of

Agriculture, Gujarat. Plants were grown and salt treatment was imposed according to the method of Prakash and Prathapasenan (1988). Briefly, plants were raised in pots lined with plastic sheets containing garden soil. Twenty seeds per pot were sown at a depth of 1 cm and were allowed to germinate. When seedlings were a week old they were thinned to 10 per pot. Pots were divided into 4 groups of 12 each for different treatments. On day 21, two groups of plants were sprayed with GA_3 (10 ppm) in 0·02% (v/v) Tween 20, the optimum concentration observed from the preliminary studies, to the point of run off prior to the imposition of salt stress. Two other groups received sprays of only 0·02% (v/v) Tween 20 solution. Salt treatment (12 dS/m) was imposed by supplying NaCl through the irrigation water on the GA_3-treated and control plants. The salinity level of the soil was maintained by checking the electrical conductivity of the soil extract periodically and by adding appropriate amount of NaCl to the irrigation water. All pots received sufficient amount of water every alternate day. GA_3 or Tween spray was repeated a month after the first spray. The maximum and minimum temperatures during the growth period were $32 \pm 3°C$ and $21 \pm 2°C$ respectively.

2.2 Growth measurements

Plants were harvested at desired intervals, washed with water, blotted and growth of the shoot system was recorded immediately. After determining extension growth, the shoot system was separated, dried at 80°C for 72 h and dry weight was determined. Growth measurements, the first two and the subsequent ones were made at an interval of 5 and 10 days respectively from the date of imposition of salt treatment.

2.3 Estimation of total chlorophyll

A known amount of fresh leaf tissue was homogenized in 80% acetone and centrifuged at 3,000 g for 10 min. The chlorophyll level in the supernatant, after appropriate dilutions, were determined spectrophotometrically according to the method of Prakash and Prathapasenan (1990).

2.4 Extraction and estimation of Na^+, K^+ and Cl^- ions

The procedure employed for the extraction of Na^+, K^+ and Cl^- ions was the one described earlier by Prakash and Prathapasenan (1988). Known weight of dried plant material was extracted thrice with boiling deionized water and the supernatant was collected by centrifuging the suspension at 6,000 g for 10 min. The residue was then extracted with 30% (v/v) nitric acid for 1 h at 90°C. The suspension was cooled and the supernatant was collected after centrifuging at 6,000 g for 10 min. The residue was re-extracted twice with 30% nitric acid. All supernatants were pooled together and made up to a known volume. Sodium and potassium ions were estimated by flame photometry and chloride by titrating with mercuric nitrate according to the method of Clark (1950).

2.5 Extraction and separation of ABA

The method employed for the extraction and purification of ABA is that of

Downton and Loveys (1978) with certain modifications. Frozen shoot or root tissue (about 10 g fresh wt.) was homogenized for 10 min in 150 ml of chilled extraction medium (methanol, ethylacetate, acetic acid; 50:50:1, v/v) containing 100 mg/l 2,6-di-tertbutyl-4-methyl phenol as an antioxidant. An internal standard of 2-trans-ABA was added at the rate of 10 μg/g fresh weight to the homogenate (Lenton *et al* 1971) and was stored in darkness for 24 h at 6°C and filtered. The residue was re-extracted twice, each time for a period of 6 h with 75 ml of the same extracting solvent. The pooled filtrates, after the addition of 35 ml of deionized water, was evaporated to aqueous phase under vacuum at 40°C. The aqueous phase was adjusted to pH 8·5 with 0·1 M NaOH and partitioned 4 times against 40 ml redistilled diethyl ether. The pH was then adjusted to 2·5 with 0·1 M HCl and the aqueous extract was partitioned 4 times against 40 ml ethyl acetate to extract the free form of ABA.

2.5a *Thin layer chromatography of free ABA*:   Extracts of free ABA were applied on glass plates coated with silica gel G (300 μm thickness) and developed in toluene:ethyl acetate:acetic acid (25:15:2). Marker spots of authentic mixed isomer ABA (Sigma, USA) were visualized under UV light without exposing the samples. The zone corresponding to authentic sample was scraped off from the chromatograms and eluted with 9:1 (v/v) acetone:methanol mixture.

2.5b *Estimation of free ABA*:   A known volume of extract, after thin layer chromatography (TLC) purification, was derivatized with BSA (bis-trimethyl silyl acetamide) as described by Davis *et al* (1968). After derivatizing with BSA 2 μl samples of extract or authentic ABA were injected into a Hewlett Packard model HP 5840 A gas chromatograph equipped with flame ionization detector and with a 50 × 0·3 cm stainless steel column packed with 10% UCW-98 on chromosorb W-AW 80/100 mesh. The column temperature was programmed from 150–250°C at the rate of 15°C min^{-1}. The injection and detector temperatures were at 250 and 300°C respectively, and the flow rate of nitrogen carrier gas was 25 ml min^{-1}. The peak area on the recorder chart was measured and the amount of ABA in the extract was determined using an ABA calibration curve. Recovery of standard ABA values presented here have been corrected for extraction and purification losses.

2.6   *Extraction and separation of gibberellin-like substances*

Extraction and purification of gibberellin (GA)-like substances were carried out according to the procedure of Jones and Lang (1968). A known weight (about 10 g fresh wt.) of plant material was frozen and homogenized with 80% methanol (100 ml) for 10 min. The homogenate was incubated at 6°C for 12 h and was filtered. The residue was re-extracted with 100 ml of 80% methanol for 6 h at room temperature and filtered. Lipid material present in the extract was removed by mixing petroleum ether (boiling range 30–60°C) with the methanolic extract. The organic solvent in the extract was evaporated on a flash evaporator at 50°C. The remaining aqueous phase was adjusted to pH 9·5 with 1 N NaOH and partitioned twice against ethyl acetate. This ethyl acetate fraction was discarded and the remaining aqueous phase was acidified to pH 2·5 with 1 N HCl and partitioned 4 times with equal volumes of ethyl acetate. The combined acidic ethyl acetate

fraction was dried over anhydrous sodium sulphate before further purification by TLC.

2.6a   *TLC of gibberellins*:   Extracts were purified by TLC prior to bioassay. Acidic fractions were reduced to dryness, redissolved in a small volume (2 ml) of ethyl acetate and streaked to the origin of 20×20 cm silica gel plates as mentioned earlier. The plates were developed in acetone: benzene: formic acid (48:50:2). Following development, the plates were divided into 10 equal zones between origin and solvent front. Each zone was scraped off and eluted 3 times with 5 ml of ethyl acetate. The eluates were reduced to dryness and redissolved in 5 ml ethanol.

2.6b   *Bioassay of gibberellins*:   Samples of TLC eluates, after evaporating ethanol, made up to a known volume with sterile distilled water and assayed with barley endosperm bioassay (Nicholls and Paleg 1963) after suitable modification. Barley seeds (selected seeds of uniform size) were soaked in a freshly prepared sterilizing solution of 5% sodium hypochlorite in a stoppered flask for 3 h at 25°C and washed 10 times with 100 ml lots of sterile distilled water. The disinfected grains were incubated in sterile distilled water for 24 h at 30°C. Seeds were then cut transversely 3 mm from the distal end with a sterile scalpel and the embryo-containing fragment discarded. The remaining endosperm portions were placed in groups of 4 each in small sterile specimen vials containing 1 ml test solution (plant extract or distilled water, pH 5·8). Each vial contained 500 μg streptomycin to prevent the contamination from microorganisms. The stoppered vials were incubated at 30°C for 48 h. All experimental manipulations were carried out under aseptic conditions. Samples of ambient media were then assayed for reducing sugars by the procedure of Somogyi (1952).

The amount of GA-like substances of the extract was calculated from standard curve prepared with authentic GA$_3$ following the above bioassay procedure. Percentage recovery of known amounts of authentic GA$_3$ standard after the extraction and purification steps detailed above was $67 \pm 3.5\%$ (mean $\pm$ SE). Amount of GA-like substances presented here has been corrected for extraction and purification losses.

2.7   Grain yield

Yield parameters analysed include total number of filled and unfilled grains, weight of filled grains per plant and weight of 1000 grains.

3.   Results

Soil salinity considerably decreased the extension growth and dry matter accumulation of the shoot system of rice (figure 1). GA$_3$ treatment, however, significantly increased the extension growth and dry weight of shoot system under saline condition. On day 60, the linear growth and dry weight of the shoot system of GA$_3$-treated salt stressed plants were, respectively, 66 and 85% more than the salt stressed plants (figure 1). Non-stressed plants also showed a significant increase in their linear growth and dry weight of shoot system as a result of GA$_3$ application.
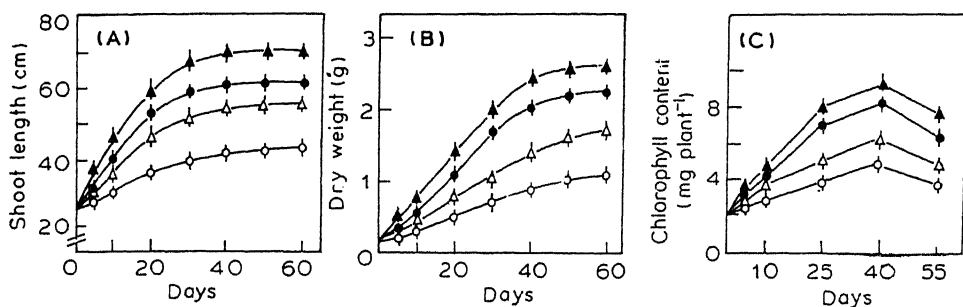
Figure 1. Effect of NaCl salinity (12 dS/m) and GA_3 (10 ppm) on extension growth (A), dry weight (B) of shoot system and total chlorophyll content (C) of rice. Vertical bars represent SE of the mean. (●), Control; (○), NaCl; (△), NaCl+GA_3; (▲), GA_3.

Changes in the chlorophyll content of plants subjected to salt and GA_3 treatments are presented in figure 1. The maximum content of chlorophyll was present on day 40 in control as well as in salinized plants. Administration of GA_3 did not change the pattern of chlorophyll accumulation either in stressed or in control plants. The total chlorophyll content of plants grown under saline condition was only about 45% of the control at the end of the experiment. However, by day 55, this level was increased by 60% more than the salt control as a result of GA_3 application. A noticeable change in the chlorophyll content was also recorded in GA_3-treated non-stressed plants compared to control.

Figure 2 shows the concentrations of Na^+, Cl^- and K^+ in the leaf tissue following salinization. During exposure to salinity, leaves accumulated very large amounts of Na^+ and Cl^- but the K^+ content was decreased. Na^+ and Cl^- ion concentrations increased with time and a major portion of these ions accumulated within 10 days of salinization. In the last determination (on day 55) the concentration of Na^+ and Cl^- in the leaves of salt stressed plants registered, respectively, 6·4- and 9·3-fold increases over the respective control values at the end of the experiment. Accumulation of Na^+ and Cl^- in the leaves was considerably limited by GA_3 application such that at the end of day 55, the levels of Na^+ and Cl^- in the leaves of GA_3-treated salinized plants were about 25 and 20%, respectively, less than that of the salt control. NaCl markedly decreased the K^+ content of leaf (figure 2) and on day 55 leaf K^+ content was 2·2 times lesser than the control plants. An appreciable increase in K^+ concentration was noticed in the leaf tissues of salt stressed plants as a result of GA_3 administration.

The content of free ABA (figure 3) in the shoot system of control plants was very low during the active phase of growth but slowly increased to higher values as the rate of growth decreased. When plants were subjected to salt stress free ABA content of shoot tissue rose rapidly for 5 days and reached its maximum level which was about 7 times more than the corresponding control value. The ABA content then declined but remained higher than that of non-salinized plants all through the experiment. GA_3 was found very effective in counteracting ABA accumulation in rice plants exposed to NaCl. Treatment of plants with GA_3 suppressed the build up of free ABA in the shoot system of salinized plants by 26% of the salt-control at the end of 55 days of salinization.

GA-like substances (figure 4) in the shoot system of control plants progressively
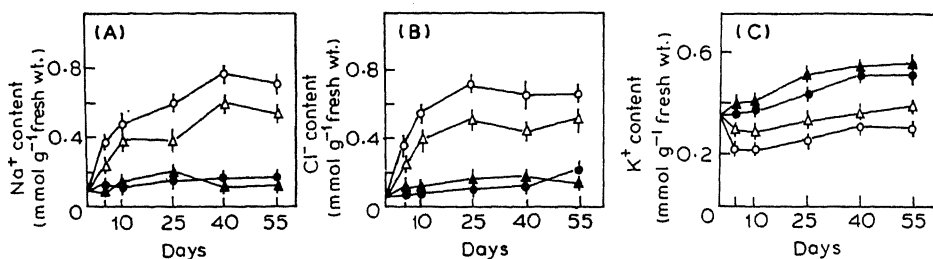
Figure 2. Effect of NaCl salinity (12 dS/m) and GA_3 (10 ppm) on Na^+ (A), Cl^- (B) and K^+ (C) content in the leaf of rice. Details of symbols as in figure 1.
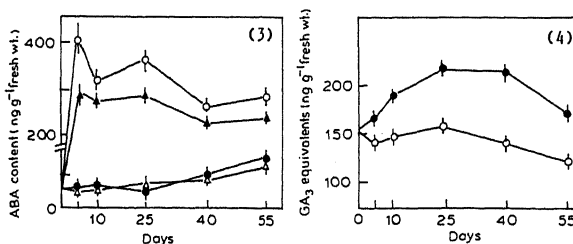
Figures 3 and 4.   3. Effect of NaCl salinity (12 dS/m) and GA_3 (10 ppm) on the endogenous level of ABA in the shoot system of rice.   **4.** Effect of NaCl salinity (12 dS/m) on the endogenous level of GA-like substances in the shoot system of rice. Details of symbols as in figure 1.

increased parallel to growth, attained its maximum content by day 25 and maintained that level until day 40. The content of GA-like substances however, considerably decreased subsequently. Salinization of the growing medium reduced the content of GA-like substances to about 70% of the control value on day 55.

There was a considerable reduction in the grain yield when rice plants were exposed to NaCl salinity (table 1). Soil salinity reduced the total number and weight of filled grains per plant and the 1000 grain weight to 26, 20 and 76% respectively, of the control values. GA_3 treatment increased the total number and weight of filled grains per plant significantly under saline condition. A slight improvement of 1000 grain weight was also discerned in salt stressed plants in response to GA_3 application.

4. Discussion

Salt stress suppressed the vegetative growth and decreased the yield output of rice. Similar observations were made on cowpea and mung bean (Balasubramaniam and Sinha 1976) and chick pea (Singh and Singh 1980) earlier. The decreased growth and yield has been found due to the low level of GA and the high concentration of ABA. It has been suggested that ABA accumulation under salt stress condition is mainly because of the altered water potential due to excess of Na^+ and Cl^- in the tissues (Wright 1978). However, no substantive evidence is available to advance a precise explanation for the reduced GA content in response to salinization. The exogenously supplied GA_3 increased the growth and yield under saline condition

Table 1. Effect of NaCl salinity (12 dS/m) and GA_3 (10 ppm) on grain yield of rice.

Treatments	Total number of filled seeds per plant	Total weight of filled seeds (g) per plant	Weight of 1000 seeds (g)
Control	152 c*	2·92 c	19·2 c
NaCl	39 a	0·57 a	14·62 a
NaCl + GA_3	72 b	1·18 b	16·4 b
GA_3	161 d	3·11 c	19·32 c

*In each column values with different letters are significantly different from each other ($P < 0.05$).

probably by counteracting the inhibitory effects of ABA and improving the hormonal balance of the plant (Scott 1984). Further, with the observations of Wignarajah *et al* (1975) and the results obtained from our earlier studies (Prakash and Prathapasenan 1990) it became increasingly apparent that NaCl inhibits growth by reducing cell division as well as cell enlargement. The stimulatory effect of GA_3 on growth and yield under saline condition observed in this study thus might also be due to its capacity to induce cell division and cell enlargement (Jones 1973).

Salt stress considerably reduced the content of chlorophyll and advanced the process of senescence in rice. This reduction in the chlorophyll content and the associated senescence can be ascribed to the massive accumulation of ABA as well as the toxic level of Na^+ and Cl^- in the leaf tissues. Reddy and Vora (1986) in their studies with wheat showed that the reduction in chlorophyll content can be attributed to the destruction of chlorophyll due to high activity of chlorophyllase. A similar observation was also made in *Cajanus indicus* and *Sesamum* grown under saline condition (Rao and Rao 1981). Application of GA_3 was found to increase the chlorophyll content under saline condition which might be a reflection of improved ionic balance as well as the low level of ABA brought about by GA_3.

A major problem facing plants exposed to salinity is the disturbances resulting from toxic levels of Na^+ and Cl^- on the physiological and biochemical processes associated with growth. Many authors have correlated high tissue concentration of Na^+ and Cl^- with decreased growth and yield of rice (Sharma 1986; Prakash and Prathapasenan 1988). Again, it seems logical to presume that besides the toxic effects caused by the accumulated Na^+ and Cl^-, the low level of K^+ in salt stressed plants will be having a direct bearing on growth and yield reduction. Apart from its role as an osmotic component, K^+ is essential for the formation of starch, protein synthesis, photosynthate partitioning, stomatal functions and above all as an activator of a number of monovalent cation requiring enzymes (Epstein 1972). While evaluating the salt tolerance mechanisms in rice varieties Sharma (1986) found that K^+ content was very much depleted in sensitive varieties and he concluded that the higher growth and yield of resistant varieties are due to better regulation over the accumulation and distribution of K^+ in the plants. Potassium content was also decreased in peanut, pigeon pea and gingelly exposed to salt stress and foliar application of K^+ partially alleviated the adverse effects of salinity on growth and yield of those crop plants (Mohan *et al* 1986).

A significant change in the ionic content, an inhibition of Na^+ and Cl^- accumulation and an increase in K^+ level, was noticed in the leaves of GA_3-treated salt stressed plants. Unfortunately GAs have not received much attention as far as their effects on ion uptake and transport are concerned. However, there are some reports to indicate that GA_3 alters the membrane permeability and regulates uptake and transport of ions (Wood and Paleg 1974). As proposed by Karmoker (1984) and from the observations of this study, it can be suggested that the inhibition of Na^+ and Cl^- accumulation and the enhancement of K^+ level found in GA_3-treated salinized plants might be due to the ability of GA_3 to alter the membrane permeability and maintain the ion uptake selectivity. Decreased influx of Na^+ and higher uptake of K^+ in response to GA_3 administration was reported by Starck and Kozinska (1980) in salt stressed bean plants. Further, they observed an increased Ca^{2+} and K^+ content in metabolically active organs in GA_3-treated NaCl stressed plants compared with control.

Application of GA_3 resulted in a considerable improvement in the yield of rice. GA_3 which is known to influence a number of processes associated with reproductive development in plants was found to antagonize the depressive effects of salinity on pollen germination in *Zea mays* (Dhingra and Varghese 1985). It is also likely that besides the inherent ability of GA_3 in influencing reproductive growth, the high concentration of K^+ in GA_3-treated salt stressed plants might have contributed towards the increase in yield output.

Acknowledgements

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Pollen analysis of *Apis cerana* and *Apis florea* honeys from Adikmet area, Hyderabad

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Abstract. Pollen analysis of 3 squeezed honey samples (one of *Apis cerana* var *indica* and two of *Apis florea*) and 160 pollen loads (100 of *Apis cerana*, 45 of *Apis florea* I and 15 of *Apis florea* II) has been carried out with a view to identify the bee forage plants and to evaluate the sources of pollen to honey bees at Adikmet area, Hyderabad.

All the 3 honey samples were found to be unifloral. While *Mangifera indica* (68·7%) formed the predominant pollen type in *Apis cerana* sample, *Tridax procumbens* (62%) and *Phoenix sylvestris* (61%) constituted the predominant pollen types in *Apis florea* I and II samples respectively. Altogether 24 pollen types referable to 20 families have been recorded. Based on the absolute pollen counts, the 3 samples are referable to group V of International Commission for Bee Botany. Out of 160 pollen loads studied, 137 were found to be unifloral, while 23 were of the mixed type.

Keywords. Pollen analysis; *Apis cerana*; *Apis florea*; honey.

1. Introduction

Plants offer pollen and nectar which serve as food to the honey bees. Nectar mostly contains sugars which serve as the sources of energy for the honey bees during their routine work, while the pollen grains referred to as 'bee bread' or 'bee meat' are the sources of proteins, vitamins, fats and minerals, of which proteins are very much essential for building the body tissues of the bees, especially during the early embryonic growth. Therefore, a knowledge of honey plants or bee plants is of importance for the growth and progress of the apiculture industry.

The bee plants can be grouped under 3 categories:
(i) Pollen sources—visited by bees for pollen alone.
(ii) Nectar sources—visited for nectar alone.
(iii) Pollen and nectar sources—visited for both pollen and nectar.

Analysis of pollen loads help us to evaluate the sources of pollen to honey bees in a locality. Similarly, pollen analysis of honey samples provide the information regarding the plants preferred by bees for nectar, as the pollen grains dispersed in honey are mostly collected by bees along with nectar. If the plants are preferred for both pollen and nectar, their pollen find their representation in both honey as well as pollen loads (Deodikar 1965; Majumdar and Chanda 1984). Suryanarayana (1986, 1987) highlighted the relevance and importance of melissopalynological studies to apiary industry and enhanced crop production.

This study is particularly designed to recognise the uni- and multifloral honeys of the Osmania University, Hyderabad area and to assess and evaluate its potential in providing nectar and pollen sources to honey bees. Studies involving pollen analysis of honey samples of Andhra Pradesh and their relevance to the bee-keeping and

honey industry in this state have been scant. Jhansi and Ramanujam (1987), recently provided an analysis of pollen types recovered from two samples of extracted and squeezed honey from Shivarampally and Kishan Bagh areas of Hyderabad. Moses *et al* (1987) evaluated the sources of pollen to honey bees at Vijayarai in Andhra Pradesh on the basis of their analysis of numerous pollen loads.

2. Materials and methods

The Osmania University campus, in the Adikmet area of Hyderabad is the source area for the honey samples and the pollen loads studied.

Three honey samples, one of *Apis cerana* var *indica* and two of *Apis florea* were collected in the month of July 1989, from the honey combs in and around the Botany Department of Osmania University. All these 3 samples represent squeezed honey. The squeezing of the honey was carried out under personal supervision and only the honey storing portion of the comb was used for this purpose. The honey thus obtained represents virtually pure honey. The honey combs of *A. florea* are seen singly in exposed areas (bushes, hedges, corners of buildings etc.); those of *A. cerana* are seen in groups of 2–7 in places with little or no light such as tree trunks or rock crevices. The composite comb of *A. cerana* in the present study was found in the more or less dark interior of a large cavity in the trunk of *Millingtonia hortensis*. Of the above 3 honey samples, that of *A. cerana* contained many dust particles.

The honey sample (1 cc) was dissolved in 10 cc of water and centrifuged. The resultant sediment was treated with 5 cc glacial acetic acid. The acetic acid was decanted and the material was subjected to acetolysis technique. Three pollen slides were prepared for each sample and were critically scanned analysing the pollen content in honeys qualitatively and quantitatively. For quantification of pollen types, 300 pollen grains were considered at random. Based on their frequencies, the pollen types recorded were placed under the following pollen frequency classes as recommended by the International Commission for Bee Botany (1970), viz., (i) predominant pollen type constituting more than 45% of the total grains counted, (ii) secondary pollen—16–45%, (iii) important minor pollen—3–15% and (iv) minor pollen—<3%. Pollen spectra and palynographs were constructed for each honey sample based on the quantification of the pollen types.

Pollen loads studied were obtained directly from the pollen chambers of the honey combs. Pollen loads (bee bread), comprising 100 from *A. cerana*, 45 from *A. florea* I and 15 from *A. florea* II combs (total of 160) were collected from the pollen storing chambers of the combs, one from each chamber. Pollen grains in each pollen load were dispersed in water and acetolysed. Two pollen slides were prepared for each load and microscopically examined. Pollen loads with one pollen type were called unifloral, with two pollen types as bifloral and loads with more than two pollen types as multifloral or mixed (Mithilesh Sharma 1970). Identification and confirmation of the pollen grains recovered from the honey and pollen loads were based upon comparison with reference slides of acetolysed pollen grains of the Osmania University campus flora.

3. Results

3.1 *Analysis of honey samples*

The 3 honey samples which were palynologically analysed were all found to be unifloral. In all, 24 pollen types referable to 20 families were recorded. Most of the pollen types recorded were common to all the 3 samples (even though their frequencies varied), indicating that the honey samples come from the same floristic region.

A. cerana honey (sample ·1) was characterised by the presence of *Mangifera indica* (Anacardiaceae, 68·7%) as the predominant pollen type. *Phoenix sylvestris* (Palmae) is the important minor pollen type of this honey. The minor pollen types are *Cucumis* sp. (Cucurbitaceae), *Loranthus longiflorus* (Loranthaceae), *Tridax procumbens* (Compositae), *Cocos nucifera* (Palmae), *Rungia repens* (Acanthaceae), *Allmania nodiflora* (Amaranthaceae), *Bombax malabaricum* (Bombacaceae), *Oldenlandia umbellata* (Rubiaceae), *Azadirachta indica* (Meliaceae), *Evolvulus alsinoides* (Convolvulaceae), *Peltophorum ferrugineum* (Caesalpiniaceae), *Ocimum* sp. (Labiatae), *Tribulus terrestris* (Zygophyllaceae), *Ailanthus excelsa* (Simarubaceae), *Ageratum conyzoides* (Compositae) and *Acacia leucophloea* (Mimosaceae).

T. procumbens (62%) and *P. sylvestris* (61%) formed the predominant pollen types in the honeys of *A. florea* I (sample 2) and II (sample 3) respectively. The important minor pollen types of *A. florea* I sample are *A. conyzoides, O. umbellata, Heliotropium zeylanicum* (Boraginaceae), *Cucumis* sp. and *Bauhinia variegata* (Caesalpiniaceae), while those of *A. florea* II are *T. procumbens, Cucumis* sp., *Allmania nodiflora, H. zeylanicum* and *R. repens. T. terrestris, A. leucophloea, Phoenix sylvestris, Citrus limon* (Rutaceae), *Peltophorum ferrugineum, Eucalyptus globulus* (Myrtaceae), *Ocimum* sp. (Labiatae), *Evolvulus alsinoides, Boerhaavia diffusa* (Nyctaginaceae) and *Randia dumetorum* (Rubiaceae) are the minor pollen types of *A. florea* I sample. *A. leucophloea, O. umbellata, T. terrestris, A. indica* and *M. indica* constitute the minor pollen types of *A. florea* II sample.

In all the 3 honey samples secondary pollen types were absent. The total number of pollen types recorded from *A. cerana, A. florea* I and II honey samples are 18 (referable to 16 families), 16 (13 families) and 11 (11 families) respectively. Fungal elements represented by spores of *Spegazzinia* and *Drechslera* and a few mycelial shreds were recorded from all the 3 samples sporadically and their percentage ranged from 0·67–1%.

The details of the palynological analysis of *A. cerana, A. florea* I and II samples are represented in table 1.

For determining the absolute pollen counts of the honey samples, the method of Suryanarayana *et al* (1981) was adopted. The absolute pollen count of *A. cerana* honey was found to be 5,37,000/g. *A. florea* I and II samples had absolute pollen counts of 2,34,000/g and 6,19,000/g respectively. According to the grading parameters of ICBB (1970) all the 3 honey samples are referable to group V.

3.2 Analysis of pollen loads

Analysis of 160 pollen loads (100 of *A. cerana*, 45 of *A. florea* I and 15 of *A. florea*

Table 1.   Pollen analysis of 3 honey samples from Adikmet area, Hyderabad.

Honey sample No.	Nature
1	Squeezed h⸱ney from a beehive of *Apis cerana* var. *indica* in the vicinity of Botany Dept., Osmania University, Hyderabad. Reddish brown in colour with absolute pollen count of 5,37,000/g. Collection date 13th July 1989
2 (*Apis florea* I)	Squeezed honey from a beehive of *Apis florea* from the Botany Dept., Osmania University, Hyderabad. Pale yellow in colour with APC 2,34,000/g. Collection date 5th July 1989
3 (*Apis florea* II)	Squeezed honey from a beehive in the vicinity of the Botany Dept., Osmania University, Hyderabad. Yellow in colour with APC 6,19,000/g. Collection date 12th July 1989
	Predominant pollen type (above 45%)
1	*Mangifera indica*
2 (*Apis florea* I)	*Tridax procumbens* (62%)
3 (*Apis florea* II)	*Phoenix sylvestris* (61%)
	Secondary pollen types (16–45%)
1	Nil
2 (*Apis florea* I)	Nil
3 (*Apis florea* II)	Nil
	Important minor pollen types (3–15%)
1	*Phoenix sylvestris*
2 (*Apis florea* I)	*Ageratum conyzoides* (9·33%), *Oldenlandia umbellata* (6%), *Heliotropium zeylanicum* (5·67%), *Cucumis* sp. (4·33%), *Bauhinia variegata* (3%)
3 (*Apis florea* II)	*Tridax procumbens* (8·67%), *Cucumis* sp. (7%), *Allmania nodiflora* (6·67%), *Heliotropium zeylanicum* (5·67%), *Rungia repens* (4·33%)
	Minor pollen types (below 3%) and fungi
1	*Cucumis* sp. (2·67%), *Loranthus longiflorus* (2·67%), *Tridax procumbens* (2·33%), *Cocos nucifera* (1·67%), *Rungia repens* (1·67%), *Azadirachta indica* (1·33%), *Allmania nodiflora* (1·33%), *Bombax malabaricum* (1·33%), *Oldenlandia umbellata* (1%), *Evolvulus alsinoides* (1%), *Peltophorum ferrugineum* (1%), *Tribulus terrestris* (1%), *Ocimum* sp. (1%), *Ailanthus excelsa* (0·67%), *Ageratum conyzoides* (0·67%), *Acacia leucophloea* (0·67%) and fungi (0·67%)
2 (*Apis florea* I)	*Tribulus terrestris* (2·33%), *Acacia leucophloea* (2%), *Phoenix sylvestris* (1%), *Citrus limon* (0·67%), *Eucalyptus globulus* (0·67%), *Peltophorum ferrugineum* (0·67%), *Ocimum* sp. (0·33%), *Evolvulus alsinoides* (0·33%), *Boerhaavia diffusa* (0·33%), *Randia dumetorum* (0·33%), and fungi (1%)
3 (*Apis florea* II)	*Acacia leucophloea* (1·33%), *Oldenlandia umbellata* (1·33%), *Tribulus terrestris* (1·33%), *Azadirachta indica* (1%), *Mangifera indica* (1%) and fungi (0·67%)

II) was carried out. As many as 137 pollen loads were found to be unifloral while 23 loads were found to be multifloral (mixed). Pollen loads strictly of bifloral nature were not encountered.

Out of 100 pollen loads of *A. cerana* studied, 95 were unifloral while 5 were multifloral. *P. sylvestris* (50 pollen loads), *M. indica* (36 pollen loads), *C. nucifera* (8 pollen loads) and *T. procumbens* (1 pollen load) are the characteristic pollen types recorded from unifloral pollen loads. Two of the mixed pollen loads had *M. indica* in high numbers (80 and 76%). *P. sylvestris*, *T. procumbens*, *C. nucifera*, *Cucumis*. sp. (3–5%); *Allmania nodiflora*, *Rungia repens*, *L. longiflorus*, *O. umbellata*, *A. conyzoides* and *T. terrestris* (below 3%) are the other pollen types recovered from these two mixed pollen loads. The pollen of *C. nucifera* were found to be in good numbers (72 and 65%) in two other mixed pollen loads. The other pollen types recovered from these include *M. indica* (10·5 and 14%), *Cucumis* sp., *L. longiflorus*, *E. alsinoides*, *A. leucophloea*, *B. malabaricum*, *A. nodiflora* and *P. ferrugineum* (below 3%). *T. procumbens* (77%) was found to be in appreciable numbers in the fifth mixed pollen load. *Ocimum* sp., *A. indica*, *C. nucifera*, *A. excelsa*, *P. sylvestris*, *R. repens*, *T. terrestris* and *A. leucophloea* (below 3%) are the other pollen types of this pollen load. The above analysis shows that *P. sylvestris* and *M. indica* constitute the major pollen source for *A. cerana* comb.

The 45 pollen loads of *A. florea* I honey sample analysed incorporate 31 unifloral and 14 mixed types. *T. procumbens* (14 pollen loads), *Cucumis* sp. (8 pollen loads), *P. sylvestris* (5 pollen loads), *A. conyzoides* (2 pollen loads), *H. zeylanicum* (1 pollen load) and *A. leucophloea* (1 pollen load) are the pollen types encountered in the unifloral pollen loads. The percentage of *T. procumbens* was found to be high in 6 mixed pollen loads (62–70%). *A. conyzoides* (5–15%), *A. leucophloea* (4–6%), *O. umbellata* (3–5%), *P. sylvestris*, *H. zeylanicum*, *B. variegata* and *Cucumis* sp. (below 3%) were also recovered from these pollen loads. Three mixed pollen loads had *A. conyzoides* in high numbers (79–83%). *T. procumbens* (6–8%), *P. ferrugineum*, *Ocimum* sp., *E. alsinoides*, *E. globulus*, *R. dumetorum* and *Cucumis* sp. (below 3%) represent the other taxa. *P. sylvestris* (77 and 63%) formed the predominant pollen type in 2 other mixed pollen loads, while *T. procumbens*, *A. conyzoides*, *B. diffusa*, *T. terrestris*, *C. limon* and *E. globulus* (below 3%) formed the minor pollen types of these two loads. The percentage of *Cucumis* sp. (77 and 65%) was found to be high in 2 mixed pollen loads. *T. procumbens*, *A. conyzoides*, *H. zeylanicum*, *Ocimum* sp. (3–15%), *P. sylvestris*, *B. variegata* and *T. terrestris* (below 3%) were the other pollen types recognised in these loads. *H. zeylanicum* (63%), *T. procumbens*, *B. variegata*, *Cucumis* sp. (3–15%), *O. umbellata*, *R. dumetorum* and *B. diffusa* (below 3%) were the pollen types recovered in the remaining (14th) mixed load. It thus becomes evident that the major pollen source for *A. florea* I comb is provided by *T. procumbens*, *Cucumis* sp. and *P. sylvestris*.

Out of the 15 pollen loads of *A. florea* II honey sample studied, 11 were found to be unifloral while 4 were of the mixed type. All the 11 unifloral pollen loads showed only *P. sylvestris* pollen. The 4 mixed pollen loads, however, showed the pollen types of *P. sylvestris* (56–69%), *T. procumbens*, *A. nodiflora*, *H. zeylanicum*, *Cucumis* sp. (3–15%), *R. repens*, *M. indica*, *A. leucophloea*, *O. umbellata*, *T. terrestris* and *A. indica* (below 3%). The pollen load analysis of the *A. florea* II comb highlights *P. sylvestris* as the major pollen source for this comb.

It may be noted significantly that the unifloral pollen loads examined show definite pigmentation based on the pollen grains they contain. The pollen loads of *P. sylvestris* are orange in colour and those of *A. conyzoides* are whitish grey. The pollen load of *H. zeylanicum* is grey in colour. The colour of the rest of the unifloral

pollen loads ranged from various shades of yellow to brown i.e., light yellow (*M. indica*), deep yellow (*C. nucifera*), mixture of orange and yellow (*A. leucophloea*), light brown (*T. procumbens*) and brown (*Cucumis* sp.).

The above qualitative and quantitative analysis of the 3 honey samples investigated is shown in the form of pollen spectra (figures 1–3). All such pollen types represented by 1% or more are incorporated in these spectra. For the pollen loads studied of each honey comb seperate composite spectra are provided (figures 4–6) to furnish information about the numerical status of diverse unifloral loads. Further, an attempt has also been made to highlight the relative percentages of the various pollen types recovered from each honey sample in the form of palynographs (figures 7–9). We define the palynograph of a honey sample as a type of polygraph which incorporates graphic depiction of its significant pollen types, resulting in a characteristic design. Both the pollen spectra and palynographs represent characteristics of the honey samples and facilitate their meaningful demarcation.
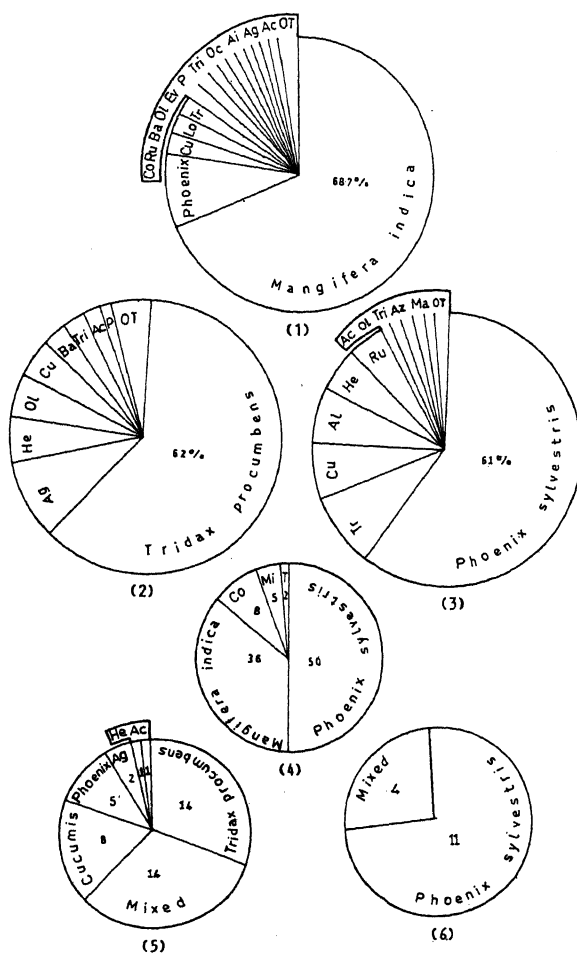
Figures 1–6. **1–3.** Pollen spectra of **(1)** *A. cerana*, **(2)** *A. florea* I and **(3)** *A. florea* II honeys of Adikmet area, Hyderabad. **4–6.** Composite spectra of **(4)** 100 pollen loads of *A. cerana*, **(5)** 45 pollen loads of *A. florea* I and **(6)** 15 pollen loads of *A. florea* II.
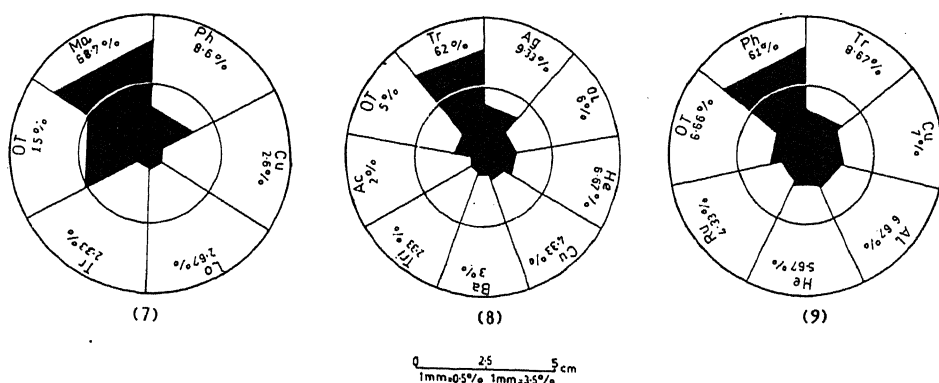
Figures 7–9. Polynographs of (7) *A. cerana*, (8) *A. florea* I and (9) *A. florea* II honeys of Adikmet area, Hyderabad.
(Abbreviations: Ac, *A. leucophloea*; Ag, *A. conyzoides*; Ai, *A. excelsa*; Al, *A. nodiflora*; Az, *A. indica*; Ba, *B. variegata*; Co, *C. nucifera*; Cu, *Cucumis* sp.; Ev, *E. alsinoides*; He, *H. zeylanicum*; Lo, *L. longiflorus*, Ma, *M. indica*; Mi, mixed pollen loads; Oc, *Ocimum* sp.; Ol, *O. umbellata*; Ot, others (include all the pollen types which are less than 1% and fungal elements); P, *P. ferrugineum*; Ph, *P. sylvestris*; Ru, *R. repens*; Tr and T, *T. procumbens*; Tri, *T. terrestris*).

Table 2 provides information on the honey bee foraging plants representing nectar and pollen source in the Adikmet area of Hyderabad.

Figures 10–35 represent the numerically significant pollen types recovered from all the 3 samples of honey investigated.

4. Discussion

The results of the pollen analysis of *A. cerana* honey and the pollen loads of its comb indicate that the foraging worker bees of this colony preferred *M. indica* as the nectar and pollen source. *P. sylvestris* and *C. nucifera* were mainly visited by bees for their pollen. *Cucumis* sp., *L. longiflorus*, *T. procumbens*, *R. repens*, *A. indica*, *A. nodiflora*, *B. malabaricum*, *O. umbellata*, *E. alsinoides*, *P. ferrugineum*, *T. terrestris*, *Ocimum* sp., *A. excelsa*, *A. conyzoides* and *A. leucophloea* constituted the minor sources of nectar and pollen to the bees of this colony. The flowering season of *M. indica* spans essentially from December to April. This obviously indicates that the bulk of nectar which constituted the source material for the honey of *A. cerana* was collected during this period (some varieties of *M. indica* are known to flower even after May). *M. indica* was also a significant source of pollen supply to the bee colony is highlighted by 36% of the unifloral pollen loads of its pollen in the comb of this honey bee. *P. sylvestris*, the flowering period of which partially overlaps (March to May) with that of *M. indica* was visited by the bees both for pollen and nectar. This is amply testified by the appreciable numbers of its unifloral pollen loads and its fairly high percentage in the honey.

T. procumbens served as a major nectar and pollen source to the bees of *A. florea* I colony. *A. conyzoides*, *O. umbellata*, *H. zeylanicum*, *Cucumis* sp., *B. variegata*, *T. terrestris*, *A. leucophloea*, *C. limon*, *P. ferrugineum*, *Ocimum* sp., *E. globulus*,

Table 2. Honey bee foraging plants providing nector and pollen source in the Adikmet area, Hyderabad.

Pollen types of bee foraging plants recorded from honey and pollen loads	*Apis cerana*		*Apis florea* I		*Apis florea* II	
	Honey	Pollen loads	Honey	Pollen loads	Honey	Pollen loads
Mangifera indica L.	+*	+*	−	−	+	+
Phoenix sylvestris (L.) Roxb	+	+*	+	+*	+*	+*
Cucumis sp L.	+	+	+	+*	+	+
Loranthus longiflorus Desv	+	+	−	−	−	−
Tridax procumbens L.	+	+	+*	+*	+	+
Cocos nucifera L.	+	+	−	−	−	−
Azadirachta indica A. Juss	+	+	−	−	+	+
Allmania nodiflora (L.) R. Br ex Wt	+	+	−	−	+	+
Rungia repens Nees	+	+	−	−	+	+
Oldenlandia umbellata L.	+	+	+	+	+	+
Evolvulus alsinoides L.	+	−	+	+	−	−
Peltophorum ferrugineum Benth	+	+	+	+	−	−
Tribulus terrestris L.	+	+	+	+	+	+
Ocimum sp L.	+	−	+	+	−	−
Ailanthus excelsa Roxb	+	+	−	−	−	−
Ageratum conyzoides L.	+	+	+	+	−	−
Acacia leucophloea (Roxb) Willd	+	+	+	+	+	+
Heliotropium zeylanicum Lam	−	−	+	+	−	+
Bauhinia variegata L.	−	−	+	+	−	−
Citrus limon (L.) Burm. f	−	−	+	−	−	−
Eucalyptus globulus Labill	−	−	+	+	−	−
Boerhaavia diffusa L.	−	−	+	+	−	−
Randia dumetorum (Retz) Poir	−	−	+	+	−	−
Bombax malabaricum Dc	+	+	−	−	−	−

+, Present; −, absent; *major source.

E. alsinoides, B. diffusa and *R. dumetorum* were also visited for both pollen and nectar while *P. sylvestris* was visited mostly for its pollen. *T. procumbens* appeared in great abundance immediately after the onset of monsoon and the honey sample of *A. florea* I is traceable to the nectar of this weed.

P. sylvestris was visited by the foraging worker bees of *A. florea* II colony for both pollen and nectar. The other taxa which served as minor sources of pollen and nectar to the bees of this colony are *T. procumbens, Cucumis* sp., *A. nodiflora, H. zeylanicum, R. repens, A. leucophloea, O. umbellata, T. terrestris, A. indica* and *M. indica.* The honey of *A. florea* II colony which is unifloral with *P. sylvestris* as the predominant pollen type when taken in conjunction with the preponderance of unifloral pollen loads of this pollen, clearly shows that it is the resultant product of the foraging activity of the bees during the summer period i.e., March to May.

From the results of pollen analysis of honey samples and pollen loads of Adikmet area, it was observed that most of the pollen types encountered in *A. cerana,* *A. florea* I and II honey samples were similar even though their frequencies varied. The foraging range, floral sources available within the foraging range, requirement of colony etc., also played an important role in determining the relative floral preferences of the bees. The occurrence of fungal elements in the honeys may be due
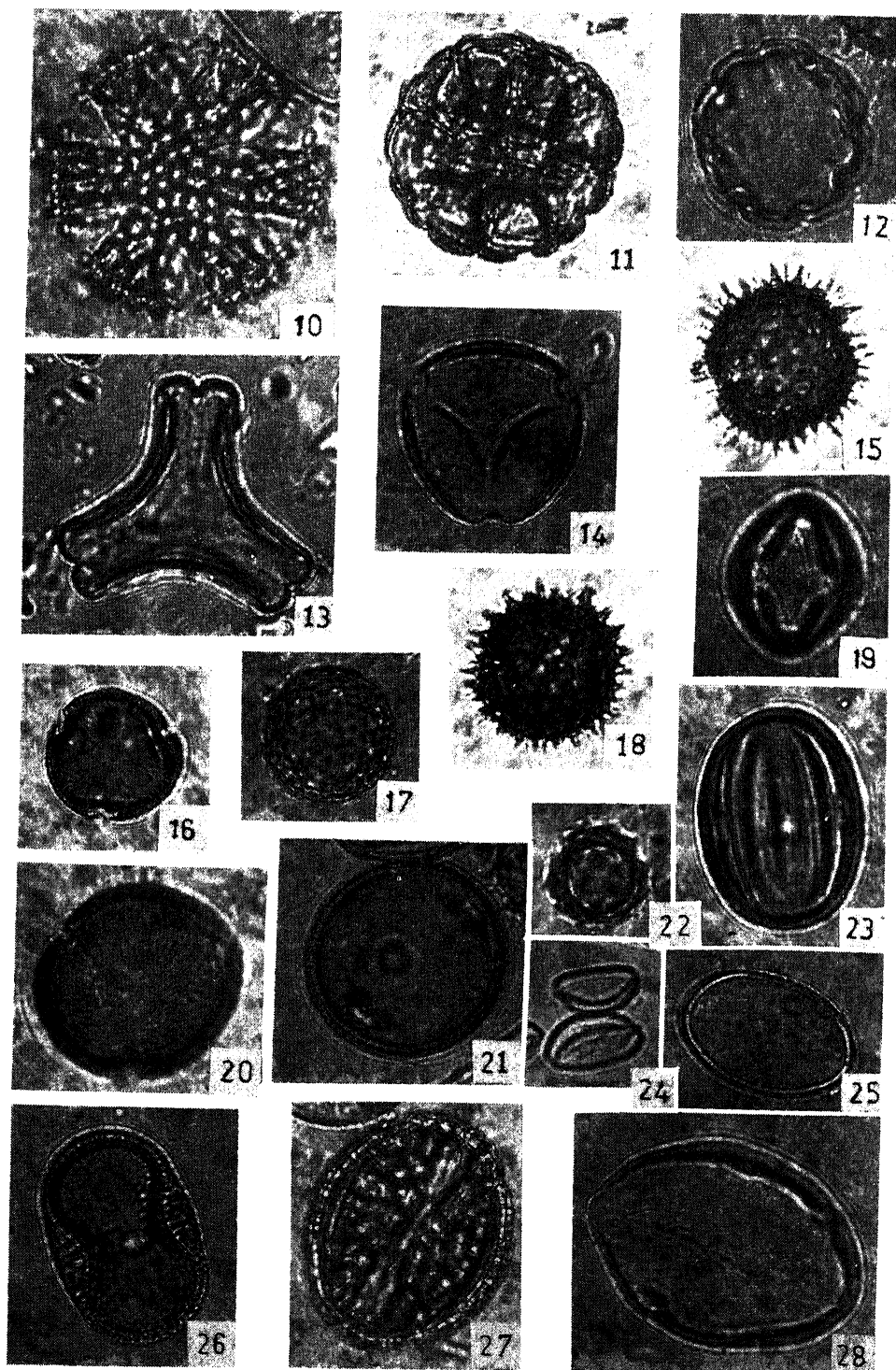
Figures 10–28. For caption, see page no. 193.
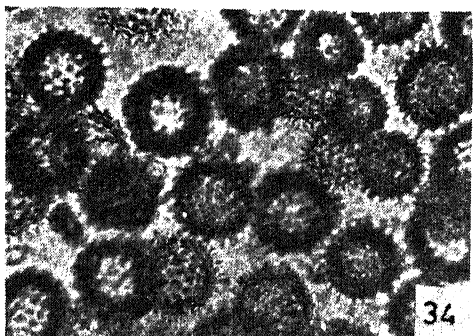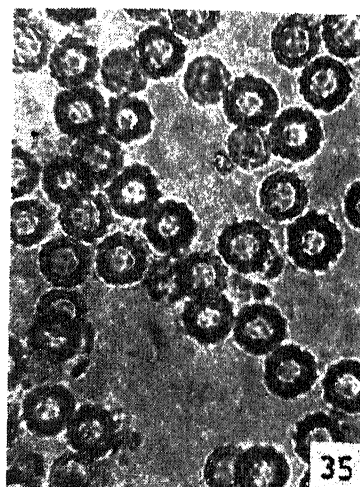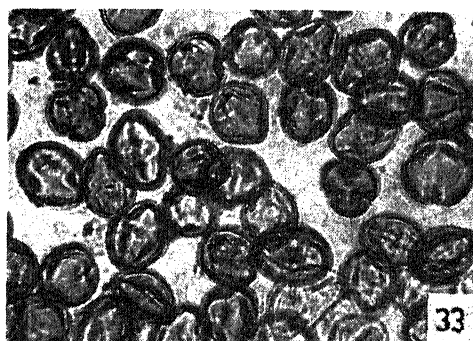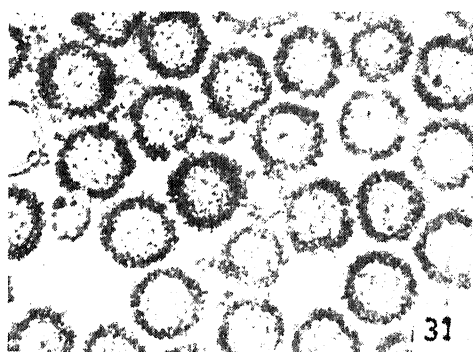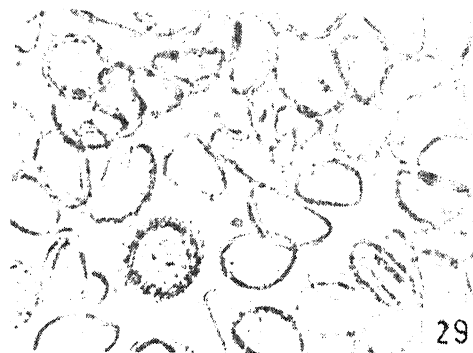
Figures 29–35. For caption, see page no. 193.

to their contamination by wind/insects or due to foraging of bees on floral nectar/pollen contaminated with fungal elements.

In conclusion it is important to note that *M. indica, P. sylvestris* and *T. procumbens* constitute the predominant sources of both nectar and pollen to the honey bees in the Adikmet area (covering Osmania University campus) during the period December to May. A number of herbaceous annuals which appeared in great profusion, as if in a flush immediately after the onset of monsoon in mid June provide minor sources of nectar and pollen to the bees during June and July.

A critical study of more number of honey samples and pollen loads from wild combs of Adikmet area should enable us not only to recognise all such key bee foraging plants constituting the essential nectar and/or pollen source to the bees all through the year but also to correctly assess and evaluate the potential of this area for the apiculture industry.

Acknowledgements

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Figures 10–28. Some significant pollen types recorded from the honeys of Adikmet area, Hyderabad (unless otherwise mentioned × 750). **10.** *Ocimum* sp. **11.** *A. leucophloea.* **12 and 23.** *H. zeylanicum.* **13.** *L. longiflorus.* **14.** *Cucumis* sp. **15 and 18.** *T. procumbens.* **16.** *O. umbellata.* **17.** *A. nodiflora.* **19.** *M. indica.* **20.** *B. variegata.* **21.** *R. dumetorum.* **22.** *A. conyzoides.* **24.** *P. sylvestris* (× 400). **25.** *P. sylvestris.* **26.** *R. repens.* **27.** *P. ferrugineum.* **28.** *C. nucifera.*

Figures 29–35. (× 400). **29–31.** Unifloral honey of (29) *A. florea* II showing *P. sylvestris, T. procumbens* and *A. nodiflora,* (30) *A. cerana* showing *M. indica* and *C. nucifera* and (31) *A. florea* I showing *T. procumbens* and *A. conyzoides.* **32–35.** Unifloral pollen load of (32) *A. florea* II showing *P. sylvestris,* (33) *A. cerana* showing *M. indica,* (34) *A. florea* I showing *T. procumbens* and (35) *A. florea* I showing *A. conyzoides.*

Pollination ecology of *Alangium lamarkii* (Alangiaceae)

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Abstract. *Alangium lamarkii* flowers during February–April. Buds are opening around the clock and offer pollen and nectar as the reward to their insect visitors. The breeding system incorporates both geitonogamy and xenogamy. Flowers are large, hermaphrodite, essential organs are centrally situated, stigma longer than anthers. Nectar contains glucose, sucrose and fructose. The glucose being dominant. Sugar concentration ranges from 25–29%. Protein and amino acids were present. Pollen-ovule ratio is 27300 : 1.

Altogether 18 insect species were found foraging at the flowers. Of the 18 species of insect foragers bees (*Apis florea, Trigona, Amegilla, Ceratina, Xylocopa latipes, Xylocopa pubescens*) and wasps (*Delta* sp., *Rhynchium*) promote both geitonogamy and xenogamy. The bees collected pollen as well as nectar. The wasps while foraging for nectar their dorsal side touches the anthers and causes nototribic pollination. The butterflies visit the flowers for nectar only, but the contact between proboscis and the essential organs is unlikely.

Keywords. Pollination; geitonogamy; xenogamy; nototriby; nectar; *Alangium lamarkii*.

1. Introduction

Pollination ecology has entered into a new phase of synthesis and correlation. A plant species in bloom needs more than one insect species for its pollination purposes. Likewise, an animal requires a series of plant species in bloom to provide continuous nourishment, then it becomes necessary as pointed out by Baker (1963) to consider the pollination relationships on a community basis. Studies of Baker (1970, 1973), Baker *et al* (1971), Frankie *et al* (1974) and Macior (1974a, b) reinforced the need for synecological study of pollination relationships. Heithaus (1974) and Frankie (1976) showed how plant-pollinator interactions are crucial in determining community structure and its functioning. The available information on pollination ecology is based mostly on the studies carried out in the temperate areas of Europe and north America. Only recently studies have been undertaken in the tropics (see Percival 1965; Proctor and Yeo 1972; Janzen 1975; Faegri and Pijl 1979). These studies pointed out the need for a synecological approach in the study of pollination (Baker *et al* 1971; Frankie and Baker 1974). In India, although some information on pollination of some tropical plants is available (Kapil 1970; Deodikar and Suryanarayana 1977; Kapil and Jain 1980; Mahrotra *et al* 1983; Reddi 1987) it is highly deplorable that even basic data on the pollination of any of the varieties of the plant species occurring is totally lacking (Mohan Ram 1980). Information on pollination of wild plants is undesirably meagre. Only recently there have been studies initiated and completed (Subba Reddi *et al* 1979, 1981, 1983; Ananthakrishnan *et al* 1981; Gopinathan *et al* 1981; Pant *et al* 1982, 1983; Subba Reddi and Reddi 1982, 1984; Reddi and Subba Reddi 1983, 1984, 1985; Birbahadur and Ramaswamy 1984; Mathur and Mohan Ram 1986; Panth and Chaturvedi

1986; Velayudhan and Annadurai 1986; Meera Bai 1987; Soloman Raju 1987; Byragi Reddy 1988; Rama Devi *et al* 1989; Jyothi *et al* 1990). The need to understand some interactions, especially in the species rich tropical ecosystem(s) is outstanding. The present study describe the interaction of 18 insect species with the flowers of *Alangium lamarkii* (Alangiaceae), a large tropical tree and also a medicinal plant.

2.   Materials and methods

A. lamarkii growing at Visakhapatnam (17° 42′ N and 82° 18′ E) was used for observations. Pollen output per anther was assessed by counting all the pollen grains in a sample obtained by gently crushing and tapping the anther on a clean microscope slide spreading the pollen mass uniformly. The longevity of pollen and stigma was based on the fruit set success from hand-pollinations at regular intervals. Nectar produced in flowers, protected from insects by butter paper bags for 2 h, was measured using disposable micropipettes. Refractometer was used to determine nectar sugar concentration. Paper chromatography was used to determine nectar sugar composition (Horborne 1973). Proteins and amino acids were identified by the method of Baker and Baker (1973). The flowers to be hand-pollinated were emasculated prior to anthesis and then bagged. Test for apomixis/autogamy, geitonogamy/xenogamy were conducted through controlled pollinations. Apomixis was tested by bagging the emasculated flowers free of pollen, autogamy by pollinating flowers with the pollen of the same flowers. Geitonogamy by pollinating flowers with the pollen of the different flowers of conspecific plant, for xenogamy with the pollen of the different conspecific plant.

Insect visitors collected during the study period at all 3 study sites (LIC quarters, Pedagadili and Simhachalam) were identified through the courtesy of Commonwealth Institute of Entomology, London and Zoological Survey of India, Calcutta. Butterflies were identified after Wynter-Blyth (1957) and their nomenclature used is that of Varshney (1983). The behaviour of visitors, the length of a visit and number of flower visits in a unit time were carefully recorded. The more frequent visitors were caught and their bodies were examined under a stereomicroscope for the pollen adhering to body areas and then washed off with alcohol.

To assess pollen amounts transferred on to the stigma in a single visit by a particular kind of insect, bagged flowers just before anthesis were opened one by one for the insects to visit. When such exposed flowers received the first visit, their stigmas were examined for pollen. Similarly the pollen deposited on the stigmas was assessed at regular intervals.

3.   Results

The plants begin to bloom soon after the cold season. The blooming season extends from February to April every year. Flowers are white or yellowish white, hermaphrodite in axillary fascicles bearing 2-3 flowers. The root bark is anthelmintic and purgative. It is useful for fevers and skin diseases, and is generally administered in the form of powder.

3.1 Phenology of anthesis

Opened flowers are evident throughout the day and night with a higher frequency during 0500-1000 h. A bud takes 20-30 min to become fully open. Concomitant with the gradual opening of flower the process of nector secretion is also started. Petals covered the stamens and stigma, but are yellowish at the time of anthesis. The petals were excited by this time and are consequently reflexed (deflexed) downwards as a sudden mechanism. They, then expose the anthers and stigmas which happens to be longer than the former to the visitors.

3.2 Flower morphology

Flowers are considerably large and hermaphrodite. Calyx tube is adnate to the ovary; limb is truncate or 4-10 toothed. Petals 4-10, linear, light green, polypetalous, valvate, thickened and recurved in flower. Flower length ranges from 1·6-2·2 cm (av. 1·8) and is 0·5 mm wide, stamens ranging from 20-30. The anthers are ditheous and introrse. Stigma is large, capitate, projecting beyond the anthers. Ovules are solitary and pendulous.

3.3 Pollen characters

Anthers open immediately after anthesis. Pollen grains are freed through longitudinal dehiscence. The number of pollen grains range from 21880-32820 ($x = 27300$) per flower. The pollen grains are spherical and small i.e., 15 μm. Exine has sculptured ornamentation, cytoplasm is granular, 2-5 colporate. Pollen grains remain viable for 40 h from the time of anther dehiscence. Pollen ovule ratio came to 27300:1.

3.4 Stigma receptivity

Stigma remains receptive for 36 h beginning with anthesis. On hand-pollination 0, 6, 12, 24 and 36 h old stigmas gave 80, 70, 60, 40 and 30% fruit set respectively and still older stigmas were not receptive.

3.5 Nectar dynamics

Nectar is situated around the ovary base. Nectar volumes measured at 2 h intervals indicating that the rate of production is not consistent throughout flower life. Sugar concentration ranges from 25-29%. The sugars present were glucose, sucrose and fructose, the glucose being dominant. Proteins and amino acids too were present. The score on histidine scale was 3.

3.6 Flower visitor activity dynamics

3.6a *Composition abundance:* During the study period, 18 insect species were found foraging at the flowers (table 1). Of these, 12 are Hymenoptera (3 Apidae; 4

Table 1.   Particulars of flower-visitors on *A. lamarkii*.

Visitor species	Forage type		Body region of pollen deposition
	Pollen	Nectar	
Hymenoptera			
Apidae			
Apis cerana indica	+	+	Head ventral side
A. florea	+	+	—″—
Trigona sp.	+	+	—″—
Anthophoridae			
Amegilla sp.	+	+	—″—
Ceratina sp.	+	+	—″—
Thyreus histrio	+	+	—″—
Pithitis bingami	+	+	—″—
Xylocopidae			
Xylocopa latipes	+	+	Head dorsal ventral side
X. pubescens	+	+	—″—
Eumenidae			
Delta sp.	−	+	Dorsal side head
Ropalidia spatulata	−	+	—″—
Rhynchium metallicum	−	+	—″—
Diptera			
Muscidae			
Musca sp.	−	+	Ventral side
Lepidoptera			
Sphingidae			
Macroglossum gyrans	−	+	Proboscis legs
Danaidae			
Danaus chrysippus	−	+	—″—
Euploea core	−	+	—″—
Pieridae			
Catopsilia pyranthe	−	+	—″—
Hesperiidae			
Pelopidas mathias	−	+	—″—

Anthophoridae, 2 Xylocopidae, 3 Eumenidae), 1 Diptera and 5 Lepidoptera (1 Sphingidae, 2 Danaidae, 1 Pieridae and 1 Hesperidae).

Of the 18 visitor species, 11 were encountered at all the 3 study sites. These species include *A. florea, Trigona* sp., *Amegilla* sp., *Ceratina* sp., *Xylocopa latipes, X. pubescens, Delta* sp., *Rhynchium metallicum, Musca* sp., *C. pyranthe* and *Pelopidas mathias*. The total number of species caught at each of the study sites was 14.

The number of visits made by different insect species at 3 study sites are given in table 2. On the whole, bees frequented most and shared 77% of the total visits followed by wasps (12·6%) and butterflies (7%).

Among each group of insects, the visits of individual species varied numerically from site to site (figure 1). Among the bee visits *Xylocopa latipes* shared 24·3% of total bee visits, followed by *X. pubescens* 24·1%, *Amegilla* 13·6%, *Ceratina* 12·2%, *Trigona* 8·6%, *A. florea* 8·8%, *A.c. indica* 4·8% and *Pithitis binghami* 1·4%. Among

Table 2. Census of flower visitors on *A. lamarkii* in 1987 season.

Insect species	LIC quarters			Pedagadili			Simhachalam		
	26/2	19/3	13/4	24/2	17/3	10/4	22/2	16/3	8/4
Bees									
Apis cerana indica	312	409	269	—	—	—	—	—	—
Apis florea	242	352	212	246	216	196	64	142	127
Trigona sp.	328	284	183	243	218	148	69	173	117
Amegilla sp.	94	194	165	682	663	409	122	188	202
Ceratina sp.	392	579	302	291	288	306	89	129	107
Thyreus histrio	—	—	—	92	86	67	86	65	73
Pithitis binghami	—	—	—	92	110	73	—	—	—
Xylocopa latipes	192	313	165	754	815	487	696	1081	473
Xylocopa pubescens	161	252	130	665	650	393	826	1422	426
Wasps									
Delta sp.	83	139	106	236	144	108	134	187	103
Ropalidia spatulata	43	85	39	—	—	—	66	218	98
Rhynchium metallicum	179	237	187	218	170	146	111	205	119
Flies									
Musca sp.	52	83	37	83	37	53	67	102	65
Moth									
Macroglossum gyrans	144	125	107	—	—	—	—	—	—
Butterflies									
Danaus chrysippus	—	—	—	—	52	49	—	48	—
Euploea core	—	—	—	88	44	33	—	—	—
Catopsilia pyranthe	27	45	55	49	32	47	86	134	107
Pelopidas mathias	66	22	45	77	134	109	110·	180	119
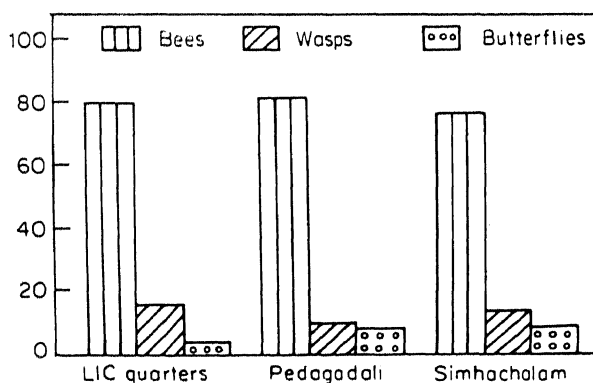
Figure 1. Insect group abundance on *A. lamarkii* flowers at 3 study sites.

the wasps the visits of *R. metallicum* was up to 46·8% followed by *Delta* sp. 36·9% and *Ropalidia spatulata* by 16·3%. Among the butterfly visits, *P. methias* shared 51·8%, followed by *C. pyranthe* 31·3%, *Euplea core* 8·9% and *Danaus chrysippus* 8%.

3.6b *Diurnal activity:* All the flower visitors are diurnal in their activity. They

appeared at the flowers during 0600–1800 h. Most of the dominant visitors exhibited a period of greater activity (*A. florea* 0800–1300 h, *Trigona* 0800–1200 h, *Amegilla* 0900–1400 h, *Ceratina* 1000–1500 h, *Xylocopa* sp. 0800–1300 h and *R. metallicum* 0830–1300 h).

3.6c *Insect behaviour at the flowers*: The bees collect pollen as well as nectar. *Amegilla* and *Apis* sp. collect pollen on their ventral side of body while touching the essential organs. *Xylocopa, Delta* and *Rhynchium* species visit the flowers for nectar and effecting the nototribic pollination. The small bodied bees such as *Trigona* and *Ceratina* concentrated on pollen collection. They alighted on the upper side of each anther and collected pollen by virtue of which their abdominal surfaces got smeared.

The butterflies visit the flowers for nectar only. The visits are sporadic. *P. mathias* and *Catopsilia* visit the flowers frequently, but the contact between the proboscis of butterfly and the essential pollinating organs of the flower is unlikely.

3.6d *Flower visits per unit time and length of a visit*: Table 3 gives the data concerning length of a visit and total flowers visited in a minute by different flower visitors. *Xylocopa* sp., *Amegilla, T. hystrio, Apis* sp. and *Rhynchium* spent relatively less time on each flower and consequently covered a large number of flowers in unit time.

3.6e *Pollen transfer in the first visit of various visitors*: The amount of pollen removed from anthers and transferred to stigma in the first visit varied with different insect species. Of the species for which such data were collected (table 4) the efficiency order is *Xylocopa, Apis, Thyreus, Amegilla, Rhynchium* etc.

3.6f *Pollen in body washings of different visitors*: The number of pollen grains found on the visitors body also depended on the body size. The *Xylocopa* sp., *Thyreus, Apis* sp. and *Amegilla* carried relatively large number of pollen on their bodies (table 5).

Table 3. Number of *A. lamarkii* flowers visited per unit time and length of visit by some flower visitors.

Flower visitor	No. of flower visits/min			Length of a visit in seconds		
	Range	Mean	SD	Range	Mean	SD
*Xylocopa latipes**	12–30	19·5	6·1	2–5	3·8	1·2
*X. pubescens**	10–30	18·0	6·0	2–6	4·0	1·0
*Rhynchium metallicum**	8–15	11·5	2·3	4–8	6·5	1·3
*Thyrius histrio**	10–20	16·0	3·4	3–6	4·3	1·0
Amegilla sp.*	15–30	22·0	6·7	2–4	3·0	1·5
*Apis florea**	8–20	13·0	3·2	3–8	6·0	1·8
*A.c. indica**	10–20	15·0	4·1	3–6	4·7	1·0
Trigona sp.*	20–30	26·0	6·0	2–3	2·6	0·5
Ceratina sp.*	20–35	34·0	8·0	2–3	2·8	0·7
*Pelopidas mathias**	1–2	2·0	0·5	30–60	48·0	11·6
*Catopsilia pyranthe**	6–9	7·0	1·2	7–10	9·0	4·0
*Danaus chrysippus**	4–7	5·0	1·0	9–15	12·0	5·0

* Sample size 10.

Table 4. Pollen depletion from anthers vs pollen deposition on stigmas of *A. lamarkii* under foragers activity.

Time (h)	No. of pollen depleted/flower	Rate of pollen depletion (%)	No. of pollen deposited per stigma	Rate of pollen deposition (%)
1000	345	31·2	27	9
1200	563	17·8	115	41
1400	760	17·0	85	28
1600	878	11·0	40	17
1800	902	1·0	15	5

Table 5. Pollen depletion from anthers vs pollen deposition on stigmas in the first visit to *A. lamarkii*.

Name of the visitor	Mean number of pollen in anthers after the visit	Pollen depletion (%)	Mean stigma pollen load after the visit	Pollen deposition (%)
Xylocopa latipes	959	13·3	30	27
X. pubescens	980	11·4	26	24
A.c. indica	1009	8·7	15	14
A. florea	1026	7·3	11	9
Thyreus histrio	1040	6·0	9	8
Amegilla sp.	1048	5·2	8	7
Rhynchium metallicum	1061	4·0	6	5
Trigona sp.	1083	3·0	2	2
Ceratina sp.	1074	2·6	4	4

Table 6. Pollen in the body washings of different forages of *A. lamarkii*.

Name of the forager	No. of pollen grains		
	Range	Mean	SD
*Xylocopa latipes**	250–510	397	95·8
*X. pubescens**	217–456	335	76·0
*A.c. indica**	87–153	123	21·9
*A. florea**	73–145	108	23·8
Amegilla sp.*	85–131	108	15·3
*Thyreus histrio**	65–109	89	14·6
Ceratina sp.*	21–45	34	9·2
Trigona sp.*	16–35	28	7·1

*Sample size 5.

3.6g *Pollen depletion from anthers vs pollen deposition on stigmas under foragers activity*: Pollen depletion from the anthers and deposition on the stigmas corresponded with the visitors' activity. During 0800–1400 h 76% pollen was removed. In the same period deposition was also high (table 6).

3.6h *Breeding systems*: Breeding experiments ruled out the possibility of

apomixis and autogamy. Out of the 50 geitomogamous flowers 68% resulted in fruit set and out of 50 xenogamous flowers 90% showed fruit set.

3.6i   *Natural fruit set*:   In open pollination 36% fruit set was observed.

4.   Discussion

4.1   *Pollination*

Opened flowers are evident at any time of the day. The flowers are hermaphrodite and homogamous. Both selfing through geitonogamy and out-crossing appear to play a role in the reproduction of *A. lamarkii* as revealed by hand-pollination experiments, but to a varying degrees. The essential parts of the flowers are placed in the centre of the blossom. The stigma is located a little above the anthers. The insects approach them with equal convenience from almost any side, and work on or from the top of them.

The bees *Amegilla, Apis* species, *Ceratina* sp., *Trigona* sp. and *Thyreus* foraged on pollen and collected the pollen through ventral side of the body, and then effected sternotribic pollination. The large bodied *Xylocopa* sp., the wasps *R. metallicum* and *Delta* sp., foraged on nectar, and then their back of head/thorex contacted the essential flower parts and received pollen nototribically. The butterflies visited the flowers for nectar only. The contact of proboscis with the essential organs are unlikely because the space between essential organs and the basal part of the flower is wide, there is no way of transferring pollen on to the stigma with proboscis.

Among the 18 flower visitors, the bees such as *A. florea, Amegilla* sp., *Ceratina* sp., *Trigona* sp., *Xylocopa* sp., the wasps *Delta* sp. and *R. metallicum* are the major pollinators (Baker *et al* 1971) because their visits are consistent and more frequent (table 2), more mobile at the flowers (table 3); picked up and transferred sufficient number of pollen. The remainder of species recorded at the flowers are to be treated as minor pollinators. Of the minor pollinators the butterflies *C. pyranthe* and *P. mathias* also made substantial visits but the contact of proboscis with the essential organs is supposedly unlikely.

Acknowledgements

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Numerical and structural inconstancy in the chromosome complements of *Belamchanda chinensis* Dc. (Iridaceae)

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Abstract. Chromosome polymorphism in *Belamchanda chinensis* Dc. of the family Iridaceae, has been explored in detail. The root tip cells exhibited aneusomaty and differences in the morphology of chromosomes. About 70% cells were with 2n = 30, 20% cells had 2n = 28 and 10% cells were with 2n = 32. Critical analysis of 3 typical cells with different chromosome numbers, selected out of several cells studied, exhibited varying proportions of long, medium and short chromosomes and different numbers of metacentric and submetacentric chromosomes. In these cells, while on one hand, all chromosomes could not be grouped in two's on the basis of chromosome morphology, certain chromosome pairs were present more than once, on the other hand. The cytological basis of inconstancy in the chromosome complements within the cells of the same tissue and it's significance in the evolution of the vegetatively propagated plants have been discussed.

Keywords. Aneusomaty; chromosome polymorphism; numerical inconstancy; structural inconstancy.

1. Introduction

In plants asexually reproducing, the chromosome complement may exhibit different degrees of inconstancy within the same tissue. Presently, while exploring certain ornamental plants cytologically, this phenomenon was noticed in *Belamchanda chinensis* Dc. of the family Iridaceae. This ornamental plant is commonly grown for it's tall spreading inflorescences and long fleshy distichous leaves. The details of the karyotype and inconstancy of chromosome complement are presented in this communication.

2. Materials and methods

Roots collected from potted plants grown in the garden of Botany Department of the University, were fixed in acetic alcohol (absolute ethanol 3 parts + glacial acetic acid one part) after pretreating them in 0·05% colchicine solution for approximately 2 h. Fixed root tips were hydrolysed in 1 N HCl at 60°C for 10 min and put in Feulgen's solution for 1 h for taking stain. Such stained root tips were squashed in a drop of 45% acetic acid. For studying chromosome morphology, camera lucida drawings were used. To analyse the karyotype, chromosomes were in 3 categories on the basis of their length: long (A), having length more than 6·8 μm, medium (B), having length between 6·8–3·8 μm and short (C), possessing length less than 3·8 μm. The chromosomes were grouped on the basis of the position of centromere as metacentric (M) and submetacentric (SM). Arm's ratio, TCl% and centromeric index (ci) were calculated using the following formulae:

$$\text{Arm's ratio} = \frac{\text{length of long arm of a chromosome}}{\text{length of short arm of the chromosome}}$$

$$\text{TCl}\% = \frac{\text{total length of a chromosome pair}}{\text{total length of the chromosome complement}} \times 100.$$

$$\text{ci} = \frac{\text{length of short arm of a chromosome}}{\text{total length of the chromosome}} \times 100.$$

Gradient index (GI) and symmetry index (SI) were calculated after Pritchard (1967), using the formulae:

$$\text{GI} = \frac{\text{length of shortest chromosome}}{\text{length of longest chromosome}} \times 100.$$

$$\text{SI} = \frac{\text{total length of all short arms}}{\text{total length of all long arms}} \times 100.$$

3. Results

The analysis of several root tips, for chromosome counts and karyotype, exhibited the presence of chromosome polymorphism and aneusomaty within the cells of root meristems. About 20% cells possessed 2n = 28 (figure 1), 70% had 2n = 30 (figure 2) and 10% cells carried 2n = 32 (figure 3). Three typical cells selected out of several cells studied were analysed critically for chromosome morphology. The idiogram of the chromosome complements present in these cells are shown in figures 4–6. Detailed information on karyotypes is given in table 1.

In all the 3 cells the chromosomes could not be grouped in two's. The cell with 2n = 28 had 12 paired and 4 unpaired chromosomes. However, 3 unpaired chromosomes A5(M), B8(SM) and C2(SM) were similar to paired chromosomes A4(M), B7(SM) and Cl(SM) respectively, in the chromosome morphology. The cell having 2n = 30 possessed 14 paired and 2 unpaired chromosomes out of which B4(SM) resembled B3(SM) pair. Similarly, the cell with 2n = 32 was having 15 paired and 2 unpaired, C8(SM) and C14(M), chromosomes resembling C7(SM) and C13(M) paired one's respectively, in chromosome morphology. However, certain chromosome pairs were represented more than once in these cells. For example, in the cell with 2n = 28, B3, B4 and B5 'pairs' possessed the same morphology; in the cell having 30 chromosomes, B7, B9, C1 and C3 were with similar morphology; similarly, in the cells with 2n = 32, C1 and C2, C7 and C8, C10–C12, C15 and C16 resembled in chromosome morphology.

A comparison of the length of chromosomes and their arms (table 1, figures 4–6) of the chromosome complements of the 3 cells, revealed the presence of highest number of A's in the cell with 2n = 28, while all the chromosomes of the cell with 32 chromosomes belong to the short (C) category. On the other hand, the cell bearing 2n = 30 exhibited the highest number of metacentric chromosomes and the cell with 2n = 32 had the maximum number of submetacentric chromosomes. The total length of chromosome complement was highest in the cell with 2n = 28, while the cell with 2n = 30 and 2n = 32 possessed the total length of complement, only, 97·56 and 62·86% respectively, in relation to the total length of chromosome complement of the cell with 28 chromosomes. Relative length of the longest
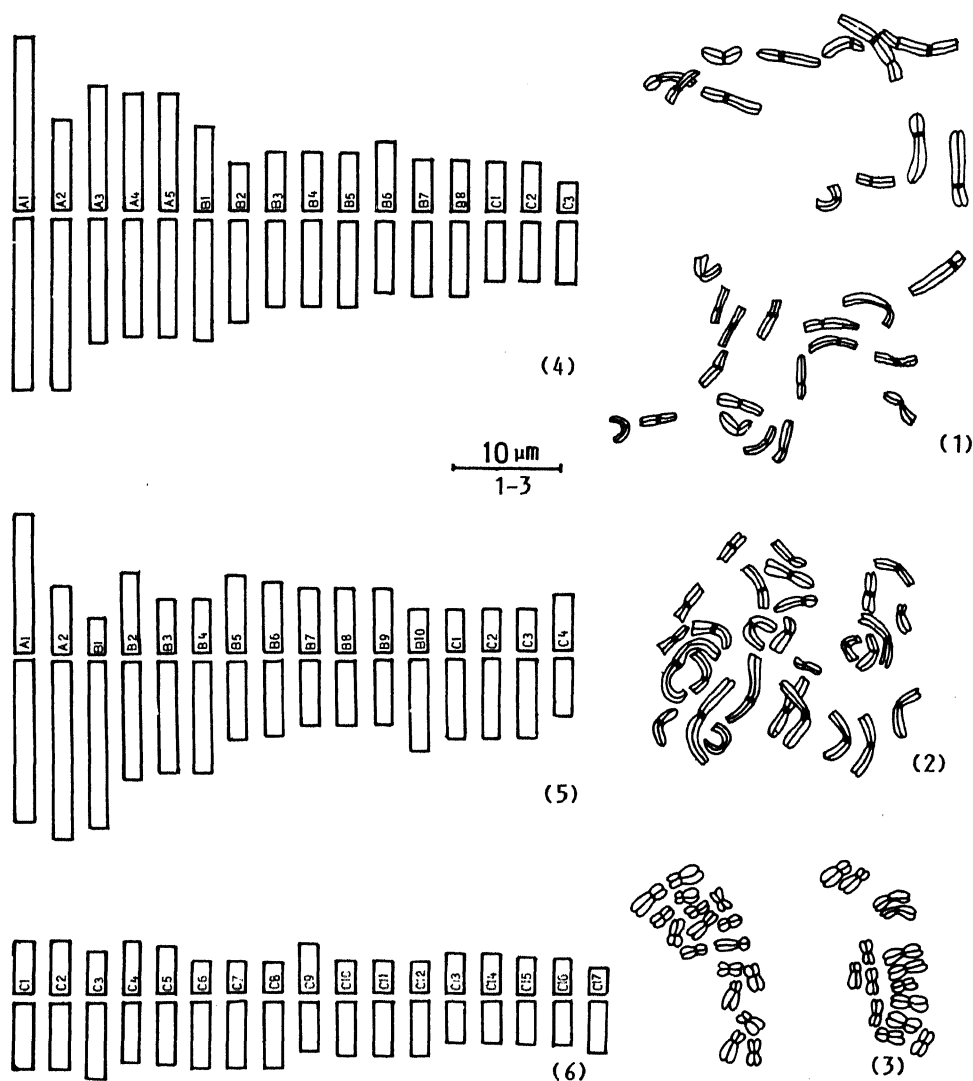
Figures 1–6. Somatic chromosomes of *B. chinensis*. **1–3.** Root tip cells with 2n = 28, 2n = 30 and 2n = 32. **4–6.** Idiograms of somatic chromosomes in figures 1–3 respectively.

chromosome of the cells having 30 and 32 chromosomes, in relation to the longest chromosome of the cell having 2n = 28, was 86·67 and 36·67% respectively. Secondary constriction was noticed only on the longest chromosome (A1, SM) of the cell having 2n = 30, on the long arm at a distance 1·9 μm from it's distal end.

4. Discussion

The root tip karyotypes of *B. chinensis*, exhibited inconstancy in it's chromosome complement. Cells with 2n = 28, 2n = 30 and 2n = 32 were present in the root tips. However, the cells with 2n = 30 were present in majority (about in 70%). The previous chromosome number reported in this plant are 2n = 32 (Nakajima 1936;

Table 1. Analysis of karyotype in the root tip cells of *B. chinensis* with 2n = 28, 2n = 30 and 2n = 32.

		Chromosome length (μm)					Relative length		
	Chromosome	Arms		Total	Arm's ratio	ci	A	B	TCl(%)
2n = 28	A1(M)	4·95	4·95	9·90	1·00	50·00	1·00	1·00	13·78
Total length of chromosome complement =									
144·34 μm	*A2(SM)	4·95	2·64	7·59	1·87	34·78	0·77	0·77	5·26
Total length of all short arms = 63·69 μm	A3(M)	3·63	3·63	7·26	1·00	50·00	0·73	0·73	10·06
Total length of all long arms = 80·65 μm	*A4(M)	3·41	3·41	6·82	1·00	50·00	0·69	0·69	9·45
GI = 28·28	*A5(M)	3·41	3·41	6·82	1·00	50·00	0·69	0·69	4·72
SI = 54·94	B1(SM)	3·49	2·47	5·96	1·41	41·44	0·60	0·60	8·26
Karyotype formula:	B2(SM)	2·97	1·48	4·45	2·00	33·26	0·45	0·45	6·16
7A(M) + 1A(SM) +	B3(SM)	2·50	1·81	4·31	1·38	41·99	0·43	0·43	5·97
2B(M) + 13B(SM) +	B4(SM)	2·50	1·81	4·31	1·38	41·99	0·43	0·43	5·97
5C(SM)	B5(SM)	2·50	1·81	4·31	1·38	41·99	0·43	0·43	5·97
	B6(M)	2·06	2·06	4·12	1·00	50·00	0·42	0·42	5·71
	B7(SM)	2·20	1·65	3·85	1·33	42·86	0·39	0·39	5·33
	*B8(SM)	2·20	1·65	3·85	1·33	42·86	0·39	0·39	2·66
	C1(SM)	1·76	1·54	3·30	1·14	46·67	0·33	0·33	4·57
	*C2(SM)	1·76	1·54	3·30	1·14	46·67	0·33	0·33	2·29
	C3(SM)	1·81	0·99	2·80	1·83	35·35	0·28	0·28	3·88
2n = 30	A1(SM)	4·62	3·96	8·58	1·67	46·15	1·00	0·87	12·18
Total length of chromosome complement =	A2(SM)	5·11	1·98	7·09	2·58	27·93	0·83	0·71	10·07
140·82 μm	B1(SM)	4·78	1·15	5·93	4·16	23·33	0·69	0·60	8·42
Total length of all short arms = 56·24 μm	B2(SM)	3·38	2·39	5·77	1·41	41·42	0·67	0·58	8·19
	B3(SM)	3·19	1·65	4·84	1·93	30·09	0·56	0·49	6·87
Total length of all long arms = 84·58 μm	*B4(SM)	3·19	1·65	4·84	1·93	30·09	0·56	0·49	3·44
GI = 40·33	*B5(M)	2·31	2·31	4·62	1·00	50·00	0·54	0·47	3·28
SI = 66·49	B6(M)	2·22	2·06	4·28	1·08	48·13	0·49	0·43	6·08
Karyotype formula:	B7(M)	1·98	1·98	3·96	1·00	50·00	0·46	0·40	5·62
4A(SM) + 9B(M) +	B8(M)	1·98	1·98	3·96	1·00	50·00	0·46	0·40	5·62
9B(SM) + 2C(M) +	B9(M)	1·98	1·98	3·96	1·00	50·00	0·46	0·40	5·62
6C(SM)	B10(SM)	2·64	1·32	3·96	2·00	33·33	0·46	0·40	5·62
	C1(SM)	2·31	1·32	3·63	1·75	36·36	0·42	0·37	5·15
	C2(SM)	2·31	1·32	3·63	1·75	36·36	0·42	0·37	5·15
	C3(SM)	2·31	1·32	3·63	1·75	36·36	0·42	0·37	5·15
	C4(M)	1·73	1·73	3·46	1·00	50·00	0·40	0·35	4·91
2n = 32									
Total length of chromosome complement =									
90·73 μm	C1(SM)	1·98	1·63	3·63	1·21	44·90	1·00	0·37	8·00
Total length of all short arms = 37·35 μm	C2(SM)	1·98	1·63	3·63	1·21	44·90	1·00	0·37	8·00
Total length of all long arms = 53·38 μm	C3(SM)	2·31	1·32	3·63	1·75	36·36	1·00	0·37	8·00
GI = 63·36	C4(SM)	1·81	1·64	3·45	1·10	47·54	0·95	0·35	7·60
SI = 69·97	C5(SM)	1·90	1·40	3·30	1·36	42·42	0·91	0·33	7·27

Table 1. (*Contd.*)

Karyotype formula:									
5C(M) + 27C(SM)	C6(SM)	1·98	0·99	2·97	2·00	33·33	0·82	0·30	6·54
	C7(SM)	1·98	0·99	2·97	2·00	33·33	0·82	0·30	6·54
	*C8(SM)	1·98	0·99	2·97	2·00	33·33	0·82	0·30	3·27
	C9(M)	1·48	1·48	2·96	1·00	50·00	0·81	0·30	6·52
	C10(SM)	1·65	0·99	2·64	1·67	37·50	0·73	0·27	5·82
	C11(SM)	1·65	0·99	2·64	1·67	37·50	0·73	0·27	5·82
	C12(SM)	1·65	0·99	2·64	1·67	37·50	0·73	0·27	5·82
	C13(M)	1·26	1·26	2·52	1·00	50·00	0·69	0·25	5·55
	*C14(M)	1·26	1·26	2·52	1·00	50·00	0·69	0·25	2·77
	C15(SM)	1·32	1·07	2·39	1·23	44·77	0·66	0·24	5·27
	C16(SM)	1·32	1·07	2·39	1·23	44·77	0·66	0·24	5·27
	C17(SM)	1·48	0·82	2·30	1·80	35·65	0·63	0·23	5·07

*Unpaired chromosomes.
A, Relative length of a chromosome in proportion to the longest chromosome of the cell.
B, Relative length of a chromosome in proportion to the longest chromosome out of all the cells.

Sharma and Talukdar 1959; Vij *et al* 1982) and n = 64 (Hsu 1971). Darlington and Wylie (1956) considered x = 8 as basic chromosome number on the basis of which the present taxon stands as tetraploid.

The karyotype of *B. chinensis* has earlier been worked out by Sharma and Talukdar (1959) and Vij *et al* (1982). Sharma and Talukdar (1959) reported the size difference between different chromosomes as 1·66–6·11 μm and 14 chromosomes with secondary constrictions. They classified the chromosomes, on the basis of size, into long, medium and short. About 7 pairs of chromosomes were reported bearing centromeres at median position and the rest at submedian position. They have also observed cells with 2n = 32 having altered karyotype in relation to the normal but did not analyse that in detail. Vij *et al* (1982) reported a size difference of 2·27–4·72 μm between the chromosomes. They observed the presence of 4 metacentric, 22 submetacentric and 6 acrocentric chromosomes. The secondary constrictions were reported present on two of the relatively larger members of the complement. The present investigation exhibited a size difference of 2·3–9·9 μm. All the chromosomes could not be grouped in two's and also, there were more than one pairs of chromosome with similar morphology. The submetacentric chromosomes out numbered the metacentric ones and acrocentric chromosomes as observed by Vij *et al* (1982), were not found. In one of the cells, having 2n = 30, only a pair of long chromosomes was bearing secondary constriction. The inconstancy in the chromosome complement was expressed in the form of numerical and structural variations. The structural variation revealed itself in the form of differences in size of the chromosomes, in the proportion of metacentric and submetacentric chromosomes and in the frequency of secondary constriction bearing chromosomes, between cells of the root tips.

After the first report of aneuploid variation of chromosomes, in somatic cells of *Paphiopedilum wardii* by Duncun in 1945, this phenomenon has been reported in several cases including *Eleutherine plicata, Cipura paludosa* (Sharma and Talukdar 1959), *Haworthia fasciata, H. subulata* (Vij *et al* 1982), *Eleusine coracana* (Maheshwari and Mann 1981), *Pennisetum americanum* (Rao and Nirmala 1986), etc. The presence of aneusomaty within a tissue can be attributed to different cytological anomalies like: (i) somatic reduction, (ii) non disjunction of chromatids,

(iii) chromosome elimination, (iv) chromosome doubling, (v) asynchronous centromere divisions, (vi) cytomixis, etc. Quite often, the chromosome mosaicism is associated with polyploidy, hybridization and apomixis. The present taxon is a tetraploid plant, commonly multiplying by vegetative means. Vij *et al* (1982) observed cells with hyperploid chromosome number in the root tips of *Haworthia fasciata* and *H. subulata*, in addition to cells with normal chromosome number $2n = 14$, and explained the absence of hypoploid chromosome number in the cells on the basis of their elimination due to disbalanced chromosome complement, since the two plants are diploids. In the case of polyploids, as is the present material, both hypoploid and hyperploid cells can enter the division cycle because of the presence of some genetic materials in duplicate.

Structural chromosomal polymorphism can result due to chromosomal aberrations like: (i) unequal reciprocal translocation, (ii) asymmetric pericentric inversions, and (iii) chromosome fragmentation. However, size difference between the chromosome complements of different cells of a tissue can also be attributed to the differential condensation of chromosomes. Structural chromosomal polymorphism within the plants of different populations have been reported in many plants including *Dolichos triflorus* (Singh and Devi 1981), *Phloex drummondi* (Madhusoodan *et al* 1982), *Vicia sativa* (Ladizinsky 1978), etc. However, the type of polymorphism noticed here along with aneusomaty within different cells of the same tissue, has been reported in some plants multiplying, either partly or entirely, through vegetative means. Sharma and Sharma (1959) emphasized the role of chromosome inconstancy, within the cells of the same tissue, in the evolution of vegetatively propagated plants, in the absence of sexual reproduction. The speciation in such plants may be effected through the entrance of cells with altered karyotype into the growing tips of daughter shoots in the course of propagation.

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Growth and alkaloid synthesis in cell lines of *Catharanthus roseus* obtained through immobilization of cells and protoplasts

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Abstract. Cells and protoplasts of *Catharanthus roseus* were immobilized with sodium alginate, agar and agarose. Cells proliferating from the matrix were established separately in liquid suspension and 5 cell lines were isolated which showed differences in their growth and alkaloid synthetic pattern. Cell lines obtained through immobilization of protoplasts yielded higher levels of alkaloids.

Keywords. *Catharanthus roseus*; immobilization; cells; protoplasts; ajmalicine.

1. Introduction

Plant cells and protoplasts have been immobilized in different matrices for investigations on secondary product biosynthesis. Under immobilized state several factors such as matrix pressure, oxygen tension, nutrient permeability, cellular exudates and cell to cell contact are known to influence the metabolic events of the cells resulting in variations of product biosynthesis (Brodelius and Nilson 1980). The immobilized cells are known to divide after prolonged period of subculture and give rise to fine suspensions comprising single cells and smaller cell aggregates (Fowler 1983). In the previous communication (Bapat *et al* 1986) we have reported the protocol for immobilization of *Catharanthus* cells and protoplasts in different matrices. The present report concerns the studies on growth and product biosynthesis of the cells emerging through immobilization matrices.

2. Materials and methods

Cells and protoplasts were immobilized in alginate, agar and agarose as described previously (Bapat *et al* 1986). For alginate immobilization cells and protoplasts were mixed with 2.5% sodium alginate (Sigma) and the mixture was pipetted dropwise into 50 ml of culture medium containing $CaCl_2$ $2H_2O$ (1·036 g/150 ml). For protoplast immobilization the osmoticum of the solution was maintained by adding 4% sucrose. The cells and protoplasts were immobilized by entrapment with molten agar or agarose medium below 30°C. After cooling the agar and agarose matrix were cut into cubes of 1 cm. The resulting alginate beads and cubes of agar and agarose were cultured in MS (Murashige and Skoog, 1962) liquid medium + 2,4-dichlorophenoxyacetic acid (1 mg/l). The cells emerging from different matrices were isolated and established on the same medium over 6 serial subcultures of 21 days. By this method 5 cell lines (i) cells immobilized in alginate, (ii) cells immobilized in agar, (iii) cells immobilized in agarose, (iv) protoplasts immobilized in agar and (v) protoplasts immobilized in agarose were established.

Growth was measured in terms of fresh weight, dry weight and packed cell volume. Packed cell volume was measured by allowing 100 ml of suspension to settle down for 20 min. The sugar content was measured on a refractometer using standard sucrose curve. For alkaloid production, cells from growth medium were transferred to production medium of Zenk *et al* (1977) using 20% inoculum and grown for 30 days.

For alkaloid analysis the tissues from growth and production medium were lyophilized, powdered and extracted by the method described previously (Benjamin *et al* 1990). Thin-layer chromatography of the basic extracts was carried out from silica gel plates using ethylacetate-methanol (96:4). The alkaloids were visualised by spraying with cericammonium sulphate and heating the plates at 110°C for 5 min. High performance liquid chromotography (HPLC) was carried out on Waters Associate Model (ALC/GPC 244) equipped with μ-bondapak C-18 column using solvent system methanol-diammonium hydrogen phosphate (70:30). Quantification of ajmalicine and serpentine was done using standard curves obtained from known concentrations of authentic samples.

3. Results and discussion

Cells immobilized in alginate beads proliferated and liberated cells into the surrounding liquid medium within 8–10 days, whereas cells entrapped in agar and agarose liberated cells into the liquid medium in 15–20 days. Active growth of the released cells occurred after 25–30 days. All the cell lines were fine suspensions comprising mainly single cell and aggregates of 10–20 cells. The cells were generally elongated and were of different sizes with prominent nuclei and dense cytoplasm (figure 1A).

Cell lines established from alginate immobilization showed an initial lag phase during 0–4 days followed by uniform growth up to 20 days and then reached a stationary phase as evidenced by fresh and dry weights. The packed cell volume reached 90% after 20 days. Most of the sucrose was utilized at the end of the eighth day (figure 1B). The cell lines derived from agar and agarose immobilization showed similar growth pattern during the incubation period of 20 days.

The parent cell line of *Catharanthus* produced trace amounts of alkaloids in MS liquid medium supplemented with 2,4-dichlorophenoxyacetic acid (1 mg/l). However, in Zenk's production medium the cells produced significantly higher levels of alkaloids of which ajmalicine was the major component. Cell lines initiated through alginate, agar and agarose immobilization of cells produced consistently lower levels of ajmalicine in Zenk's production medium as compared to the parent cell line (table 1).

Protoplasts immobilized in alginate failed to divide. However, protoplasts entrapped in agar or agarose divided and liberated cells into the surrounding liquid medium at the end of 8–10 weeks. The colonies comprised single cells and aggregates of 10–20 cells. Morphologically these cell lines were similar to those derived from cell immobilization. The cell line initiated from the protoplast immobilization showed uniform growth from the 4th day and reached stationary phase on the 12th day as evidenced by dry weight and packed cell volume increment. HPLC analysis of the basic extract of the cell lines derived through
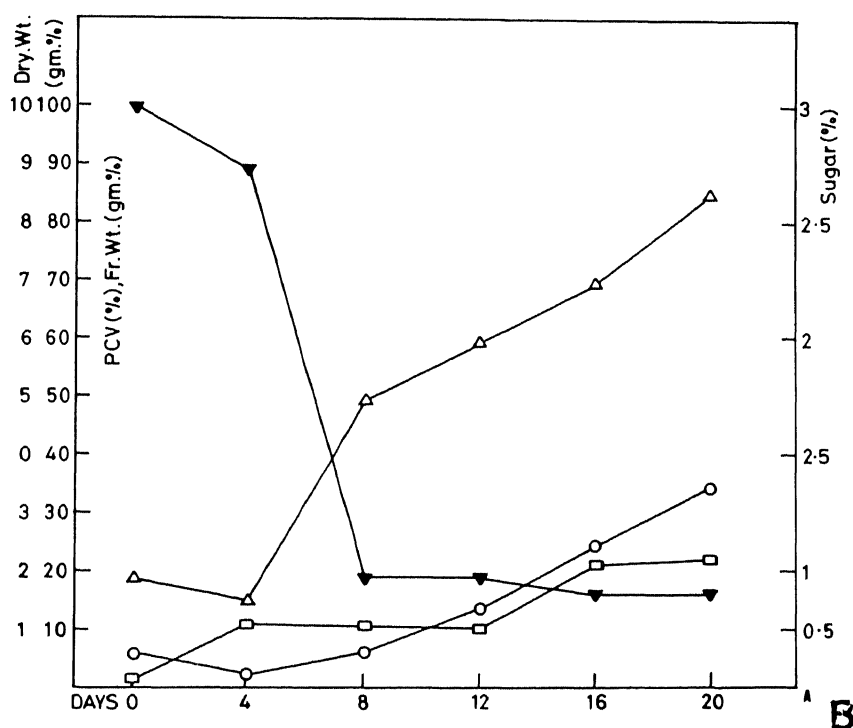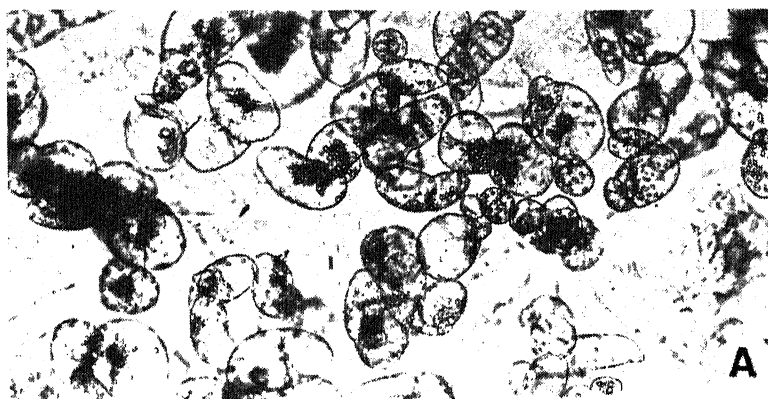
Figure 1. A. Cells from suspensions of *C. roseus* derived from immobilized cells. B. Growth of *C. roseus* cell suspension derived from immobilized cells in alginate. (△), Packed cell volume; (▼), sucrose; (○), fresh weight; (□), dry weight.

immobilization of protoplasts showed that ajmalicine content was more in these cells. Small amounts of serpentine was also present in these extracts (table 1).

The 5 cell lines of *Catharanthus* in the present study derived through immobilization of cells as well as the protoplasts exhibited marked changes in their biosynthetic activities which could be attributed to the effects of immobilization and protoplasts isolation. Cell lines derived through immobilization of cells in alginate,

Table 1. Alkaloid content of cell lines derived from immobilization of cells and protoplasts of *C. roseus*.

Treatment	Cells		Protoplasts	
	Ajmalicine (mg/100 g/dw)	Serpentine (mg/100 g/dw)	Ajmalicine (mg/100 g/dw)	Serpentine (mg/100 g/dw)
Control	123	—	—	—
Alginate	52	1·3	—	—
Agar	66	—	140	0·4
Agarose	30	—	170	7·9

The cells were grown in production medium of Zenk, with a 20% inoculum. dw, Dry weight.

agar and agarose gave consistently lower yield of ajmalicine as compared to the parent. On the other hand cell lines obtained through protoplasts immobilization in agar and agarose produced higher levels of alkaloids as compared to the parent cell line. Cell lines derived through immobilization have been shown to differ in their extent of aggregation and morphology. However, there is no report on the metabolic status of such cells. The present data clearly demonstrates that immobilization of cells and protoplasts bring about marked changes in the alkaloid synthetic pattern of cells emerging through the matrix.

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Anatomy of the stems of seedling palms

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Abstract. The anatomy of stem in 19 species of seedling palms is described in representative species from all the sub-families of Palmae, except Nypoideae and Phytelephantoideae. Morphologically, the stem of juvenile palm is an obconical structure, and that of the adult palms mostly solitary columnar. The cortex of stems in young palms is very wide, and often exceeds the diameter of central cylinder in contrast to very narrow cortex in adult palms. The number of vascular bundles increases several-times from the lower to the upper level of juvenile axis, whereas the number of bundles more or less, remains the same at different levels of the stem in adult palms. There is a wide meristematic zone towards the tip of axis and just below the bases of the young leaves which is responsible for widening of the seedling stem until it attains the mature stem diameter. The xylem of central vascular bundles in young palms is mostly composed of protoxylem elements but, in adult palms, they include well-developed metaxylem vessels. The central ground parenchyma is compact in young palms, spongy and lacunose in adult palms.

Keywords. Anatomy; stem; meristematic cap; palms.

1. Introduction

The family palmae comprises about 2,800 species in over 200 genera (Moore 1973). An admirable study on the anatomy of vegetative organs of 250 adult species is by Tomlinson (1961). His investigation is chiefly confined to lamina, although in some species he has studied the stem and root as well. The literature on the anatomy of stems of seedling palms is very limited (Helm 1936; Tomlinson and Zimmermann 1966; Davis *et al* 1975, 1978). The present anatomical investigation on the stems of 19 species of seedling palms attempts to compare and contrast the structure of young and adult palms.

2. Materials and methods

This present investigation deals with the stem of following 19 species of seedling palms:

Sub-families	Species	Age (months)
Coryphoideae	-*Livistona rotundifolia* (Lam.) Mart.	10
	Rhapis excelsa (Thunb.) Henry	12 (sucker)
Phoenicoideae	-*Phoenix reclinata* Jacq.	8
	P. rupicola Anders.	10
	P. sylvestris (L.) Roxb.	16
	P. pusilla Trimen	16

Borassoideae	-*Borassus flabellifer* L.	8
	Hyphaene dichotoma (white) Furtado	15
Lepidocaryoideae	-*Calamus tenuis* Roxb.	12 (sucker)
	Salacca zalacca Reinw.	12
Caryotoideae	-*Arenga pinnata* (Wurmb) Merr.	30
	Caryota urens L.	12
Arecoideae	-*Areca catechu* L.	24
	A. triandra Roxb.	19
	Chrysalidocarpus lutescens H A Wendl.	16
	Roystonea regia (H B K) cook	11
	Veitchia merrillii (Becc) Moore	10
Cocoideae	-*Cocos nucifera* L.	12
	Elaeis guineensis Jacq.	12

The seedlings were mostly raised at the Crop Garden of the Indian Statistical Institute, Calcutta. According to their availability, the seeds were sown at different intervals, for a few species, seeds were not available, and investigations are limited only to Ca 1-year old suckers: *C. tenuis* and *R. excelsa*.

As the stems of seedlings are rather small, the material was cut vertically into two or four equal pieces, and fixed in FAA. After 12 h, to soften and desilicify the tissue, it was soaked in commercial (48%) hydrofluoric acid and glycerol-alcohol (10% glycerol, 70% ethanol) for 4 to 5 days (Uhl 1969). The treated pieces were washed in running water for about 8 h, dehydrated with graded ethanol series, infiltrated with chloroform-paraffin wax, embedded in wax, 14–22 μm transverse sections were cut at different levels from apex to base, and stained in safranin and fast-green.

3. Results

3.1 *General anatomy*

The stems of palm seedlings are obconical, but those of adult palms are variable, mostly columnar (figure 1).

In a seedling stem the epidermis is more or less uniform, usually cutinized, and comprises isodiametric or cubical cells. The epidermal cells are slightly columnar, e.g., in *Chrysalidocarpus* and *Roystonea*. Trichomes are rare (e.g., *Caryota*). Stomata are present in some plants, e.g., in *Areca* and *Rhapis*. Each guard cell has two conspicuous ledges. The sub-stomatal chamber is absent. The hypodermis is inconspicuous. The cortex is very wide at the lowest level of stem, may be as much as the radius, or more, of the central cylinder (figure 2). It becomes narrower at higher levels. The cortex is largely made up of ground parenchyma cells containing numerous fibrous strands (figures 3–5), and a few vascular bundles, some bundles are inversely oriented. The peripheral cortical cells are smaller than the ground parenchyma of central cylinder, e.g., in *Areca*. A ligno-suberized cork sometimes occurs on the outer cortex, and below this zone is an etagen type of meristem in *A. triandra* (figure 4). The cortex includes numerous longitudinal fibrous strands. Frequent horizontal leaf traces often pass from the cortex to the periphery of central cylinder (figure 3). A few radially arranged parenchyma cells are present in the outer peripheral region of cortex (e.g., *Areca*), or in the middle cortex (e.g.,
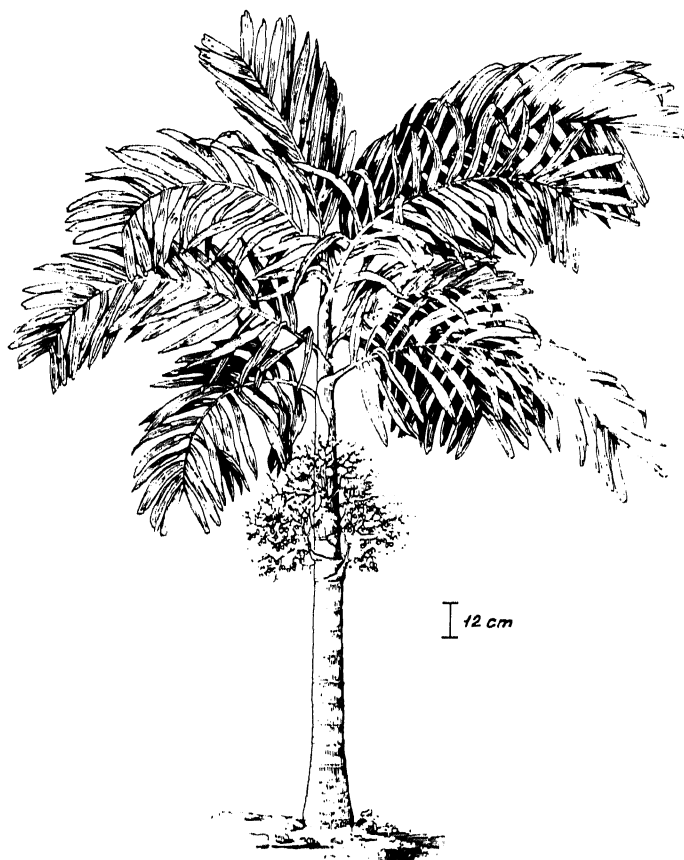
Figure 1. *A. catechu*, adult plant with columnar stem.

Cocos), and are tangentially expanded. Cortical parenchyma cells often contain abundant starch grains (figures 4, 5) tannin cells, and raphide sacs. Stegmata (silica cells) are present adjacent to each fibrous strand, and fibrous phloem sheath of the cortical vascular bundles which may be spherical (*Phoenix*) or hat-shaped (*Arenga*).

The central cylinder can be distinguished from the cortex by a peripheral zone of congested vascular bundles (figures 2, 7, 8). Sometimes more than two peripheral vascular bundles become confluent, as in *Elaeis* (figure 7) and *Rhapis*. Usually, a layer of cells does not separate the cortex from the central cylinder but, in some palms, like *Cocos*, a sclerotic narrow discontinuous zone (4–6 layers) occurs 10·5 mm above the base of stem. The sclerotic cells are tangentially extended, and widely pitted; 10·2 mm from the base the sclerotic zone disappears. The sclerotic zone seems to be a large root-trace. At higher levels towards the tip of the axis and just below the bases of young leaves, a wide meristematic zone (figure 6) occurs in a semi-convex manner. This meristematic zone is responsible for widening of the stem in the seedlings until it attains the mature stem diameter. Within this zone some procambial strands run almost horizontally which form the future vascular bundles. The shoot apex proper is very small. During early development the stem diameter increases progressively, so that each successive inter-node becomes wider than the
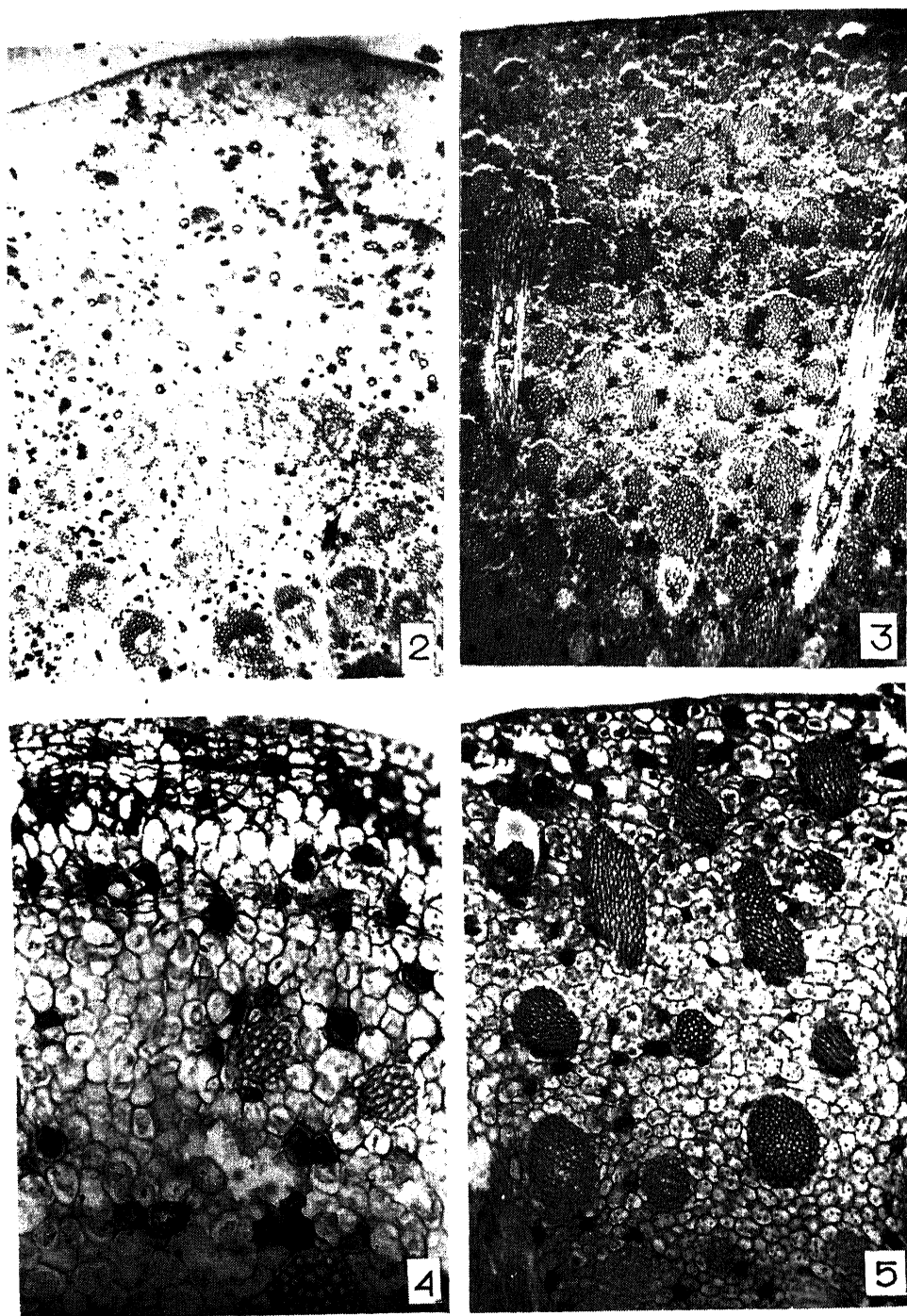
Figures 2–5. Transections of stems. **2.** *E. guineensis*, cortex and part of central cylinder (× 32). **3.** *P. sylvestris*, cortex (× 40). **4.** *A. triandra*, cortex, note 'etagen' phellogen at peripheral region (× 112). **5.** *L. rotundifolia*, cortex (× 82).
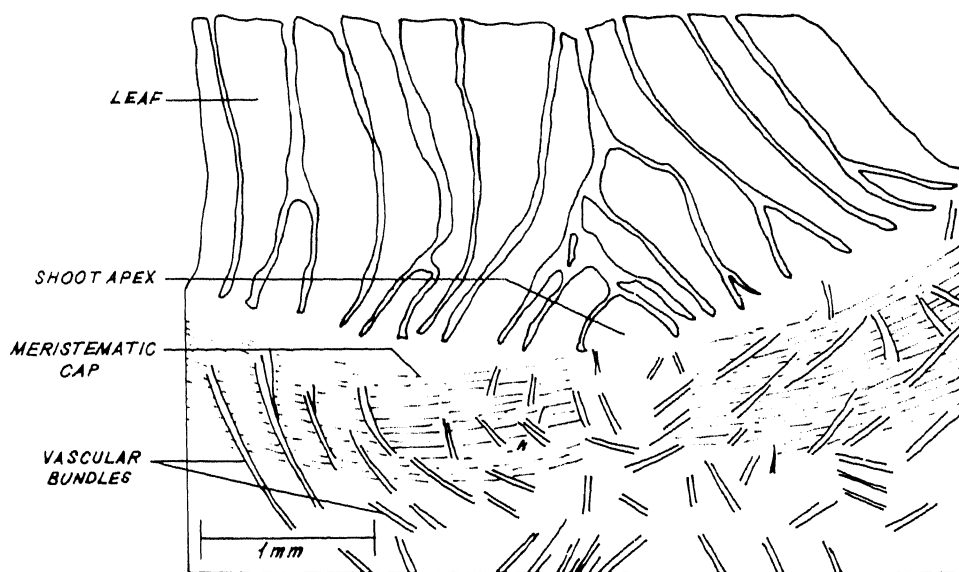
Figure 6. *P. sylvestris*, median longitudinal section of stem showing meristematic cap.

preceding one. Due to this type of early development the seedling stem has an obconical shape. Each peripheral vascular bundle has a moderately-wide fibrous phloem sheath. The phloem is usually narrow. The xylem is sheathed by slightly sclerotic parenchyma cells, and has 1–3 comparatively large and a few small vessels, mostly of protoxylem origin. The peripheral vascular bundles are smaller, and more congested than diffusely distributed large central bundles (figures 7, 8). Some leaf-traces pass from the periphery of the cylinder to the cortex; a few traces also occur at the centre of stem. Root traces at the peripheral region of central cylinder are common (figure 7). Rarely, a few peripheral vascular bundles develop obliquely, or are inversely oriented (e.g., *Borassus*). The number of vascular bundles rapidly increases from a few bundles at the lower-most level of the axis to as many as 260 bundles (e.g., *A. catechu*) at the higher level, just a few mm above the obconical base of stem. Central vascular bundles are scattered, circular in transection, large, and each has a well-developed fibrous phloem sheath; the xylem is surrounded by parenchyma cells (figures 7, 8). Metaxylem vessels are inconspicuous, narrow, and usually indistinct from the protoxylem vessels (figure 9). Protoxylem is well-developed, becomes more pronounced at the uppermost level, just before the bundles enter into leaf. The phloem strand is usually undivided but, sometimes, they divide into two separate strands by a narrow median sclerotic partition (e.g., *Calamus*). According to the composition of xylem and phloem of the central vascular bundles, 5 categories are recognized in the species investigated:

(i) Vascular bundles mostly with one wide vessel; phloem strand undivided—*R. excelsa*.

(ii) Vascular bundles mostly with one wide metaxylem vessel; phloem strand divided into two separate strands—*C. tenuis*.

(iii) Vascular bundles mostly with two inconspicuous, wide vessels; phloem strand undivided (figures 7, 8)—*A. catechu*, *A. triandra*, *A. pinnata* and *E. guineensis*.
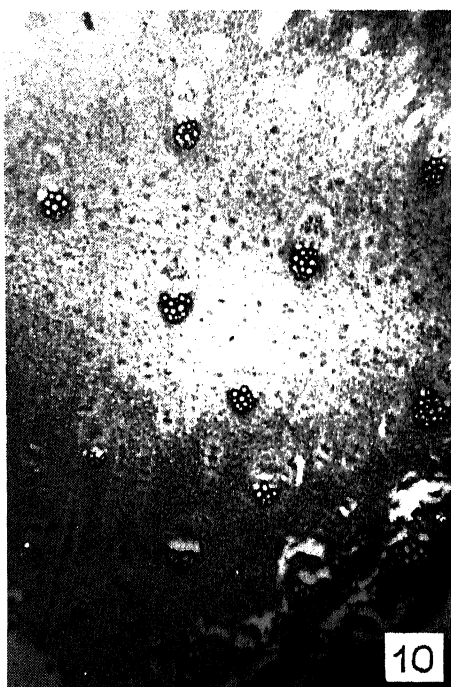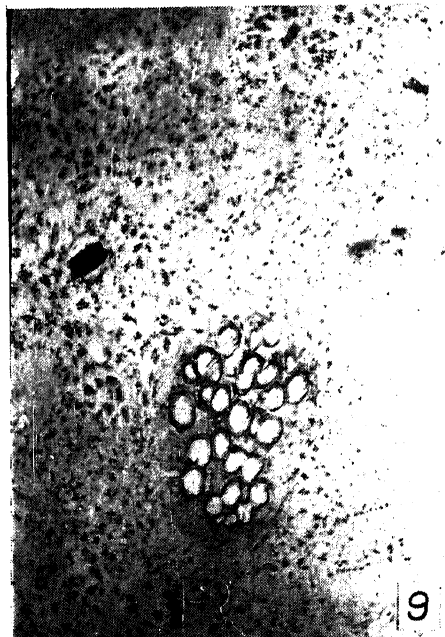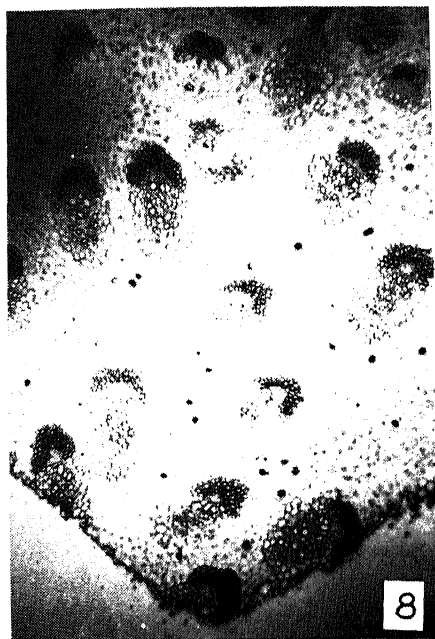
Figures 7–10. Transections of stems. 7. *E. guineensis*, part of central cylinder (×85). **8.** *A. catechu*, centre of stem (×40). **9.** *B. flabellifer*, vascular bundle from centre of stem; also note raphide sac (×78). **10.** *P. sylvestris*, part of central cylinder (×40).

(iv) Vascular bundles mostly with narrow protoxylem vessels; phloem strand undivided (figure 10)—*C. urens, C. lutescens, C. nucifera, L. rotundifolia, P. reclinata, P. rupicola, P. sylvestris, P. zeylanica, R. regia, Salacca zalacca* and *V. merrillii.*

(v) Vascular bundles mostly with more than two wide protoxylem vessels; phloem strand undivided (figure 9)—*B. flabellifer* and *Hyphaene indica.*

The ground tissue of central cylinder is parenchymatous, more or less uniform and compact, including starch grains, tannin cells, and frequent raphide sacs. Air canals are absent. Fibrous strands are usually absent in the central cylinder, although they are occasionally present in *Cocos* and *Salacca.*

3.2 *Chief anatomical differences between young and adult palms*

Young plants	Adult plants
The stem is obconical	Mostly columnar (Tomlinson 1961)
The cortex is very wide, often exceeding the diameter of central cylinder	The cortex is very narrow
The etagen-type meristem in outer cortex observed only in *A. triandra*	The etagen-type meristem reported in *A. catechu, Chamaedorea, Metroxylon, Rhaphea* and in many other plams
The xylem of central vascular bundles comprises protoxylem elements or, rarely, narrow metaxylem vessels	The xylem of central vascular bundles contains one or more wide, dominant metaxylem vessels
The number of vascular bundles increases from the lower level to the upper level	The number of vascular bundles per unit area of stem is approximately the same at all levels
The ground parenchyma tissue is compact	The ground parenchyma tissue is lacunose

4. Discussion

Morphologically a seedling of a palm differs in many ways from its adult predecessor. For example, the leaves of seedlings are usually simple where as in adult they are palmate or pinnately compound (Tomlinson 1960). Similarly the stem of adult palms is mostly columnar and it is obconical in young plants.

The internal structure of stems of young palms shows variable characters quite different from that of adult axis. The formation of an obconical axis in the seedling is caused by a meristematic cap (Zimmermann and Tomlinson 1970) just below the apex and leaf bases. At the seedling stage the function of this cap is only to add cells laterally causing to form a wide stem base, and vertical elongation occurs when the base attains its mature width. This growth in diameter is progressive, that means each successive internode becomes wider than the preceding one, causing an obconical shape. The establishment of mature girth at an early age is produced by the activity of a primary thickening meristem (Schoute 1912; Helm 1936; Ball 1941; Eckardt 1941), and the subsequent elongation of the trunk occurs by the maturation of cells below the apex. The gradual increase in girth of the stem has been termed establishment growth (Zimmermann and Tomlinson 1970; Tomlinson and Esler 1973).

The abundance of protoxylem elements and the presence of a few narrow metaxylem elements in the seedling stems in contrast to wider metaxylem elements in adult axis is an interesting finding. Wide metaxylem vessel elements are quite common in roots of both seedlings and adult plants (Ghose 1984, 1987). Hence, it supports the view of Cheadle (1943) that the vessels first originate in the root and only later in the stem and leaf.

The compact ground parenchyma tissue in the central cylinder of the seedling stem is another contrasting character with the adult axis where the initially isodiametric cells elongate and separate to form the large intercellular spaces (schizogenous lacunae) which are responsible for the sponginess of the central zone in the mature axis (Mohl 1824; Zodda 1904; Schoute 1912; Tomlinson 1961; Waterhouse and Quinn 1978). According to Zodda (1904), two phases of growth are involved—a phase of cell-division preceded that of cell expansion. This type of growth in the adult axis has been termed as diffuse secondary growth by Schoute (1912) and Tomlinson (1961). Monoyer (1925) called it as sustained primary growth. Hence, as the diffuse secondary growth occurs only in the adult palms, spongy parenchyma of the ground tissue is absent in the seedling stems.

Acknowledgement

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Light activation of enzymes in relation to leaf age in *Vigna unguiculata* (L.) Walp and *Zea mays* L.

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Abstract. The effects of light-dark transition and aplastidic condition by the photobleaching herbicide, 3-amino-1,2,4-triazole, on the activities of enzymes like *in vivo* nitrate reductase, peroxidase, polyphenol oxidase and catalase were studied in leaves of different ages of 30-day old plants of *Vigna unguiculata* and *Zea mays*. The activity of nitrate reductase was found to be higher in young leaves and showed a gradual decline at the time of maturity and senescence. In amitrole-bleached young leaves, *in vivo* nitrate reductase activity was significantly reduced. Although peroxidase is a light-activated enzyme, the enzyme was more active only in fully mature and senescing leaves of *Vigna unguiculata*. In *Zea mays*, peroxidase activity was more in mature leaves compared to senescent leaves. Interesting feature of this enzyme was that its activity increased upon dark treatment in *Vigna unguiculata*. A similar trend was also observed in polyphenol oxidase activity in mature and senescent leaves of *Vigna unguiculata* and *Zea mays*. Foliar spray of amitrole increased peroxidase and polyphenol oxidase activities in the young leaves of *Zea mays* and *Vigna unquiculata*. The results are discussed in relation to age of leaves and the presence or absence of leaf plastids.

Keywords. Nitrate reductase; peroxidase; catalase; *Zea mays*; polyphenol oxidase; amitrole; *Vigna unguiculata*.

1. Introduction

Light regulation of chloroplast development in higher plants involves complicated systems in which atleast 3 photoreceptors, i.e., phytochrome, protochlorophyllide and a blue light receptor operate. These receptors may work in tandem, sequentially (Mohr 1986) or, perhaps, competitively depending on light quality and irradiance level. Leaves are efficient light filters, absorbing red and transmitting far-red.

Light modulates the activities of several chloroplast enzymes. The chloroplastic NADP-malate dehydrogenase of both C_3 and C_4 plants is one such enzyme which is activated by light and deactivated in the dark (Johnson and Hatch 1970; Scheibe and Beck 1979; Edwards *et al* 1985). In recent times, there have been several reports on light activation of enzymes (Ashton and Hatch 1983; Nakamoto and Edwards 1983; Scheibe *et al* 1986; Vivekanandan and Edwards 1987). In the present study to measure the levels of light-activation and dark deactivation of a few enzymes like nitrate reductase, peroxidase, polyphenol oxidase and catalase, light-grown plants at different periods of growth were taken. The effect of ageing on the levels of enzymes in relation to chloroplast development was also investigated.

As another aspect of the present study, the herbicide, 3-amino-1,2,4-triazole (amitrole) was used which has been reported to affect the chloroplast development specifically without any significant effect on the rest of the cellular metabolism (Gnanam *et al* 1974; Vivekanandan and Gnanam 1975b). Hence in the present study, the herbicide, amitrole has been used as a foliar spray at different ages of

growth on light-grown plants to find out the effect of amitrole-induced absence of chloroplasts on enzyme activity.

2. Materials and methods

Seeds of *Zea mays* L. and *Vigna unguiculata* (L.) Walp were selected for uniformity and planted in earthen pots ($33 \times 33 \times 30$ cm; 5 or 6 plants per pot) filled with red sandy loam soil in the ratio of $1:7$ (1 kg sand + 7 kg red loamy soil). Healthy and disease free seeds were selected and soaked for 12 h overnight in tap water and then sown in different pots. The potted plants were grown in direct sunlight with daily irrigation. For dark treatment, the light-grown plants were kept in a light-proof dark room for 3 h. A low intensity green safe light was used for collection of leaf samples and homogenization of leaf tissue in dark (Nakamoto and Edwards 1983).

The assay of enzymes was carried out in leaves of different ages on different nodes (from top to bottom).

<div align="center">

Z. mays

</div>

Young leaves	Node 1
Moderately mature leaves	Node 3
Mature leaves	Node 4
Senescent leaves	Node 5

In *V. unguiculata* leaves on all the 4 nodes (from the top to the bottom) were selected for experiments.

Thirty-day old light-grown *Z. mays* and *V. unguiculata* seedlings were sprayed with aqueous solution of 2 different sub-lethal concentrations (2·5 and 5 mM) of amitrole. For assay of enzyme activities amitrole-sprayed green, partially bleached, totally bleached and senescent leaves were selected to find out the influence of absence as well as senescing chloroplast on the activities of a few enzymes envisaged in the present study. Water sprayed plants of similar age were treated as controls.

2.1 *Assay of in vivo nitrate reductase, peroxidase, polyphenol oxidase and catalase activities*

In vivo nitrate reductase activity was determined by Hageman and Hucklesby (1971). Freshly harvested leaves were uniformly segmented or leaf discs prepared and vacuum infiltrated. After infiltration, the vials were incubated for 30 min in *Z. mays* and 120 min in *V. unguiculata* in dark at 30°C. Peroxidase and Polyphenol oxidase activities were calculated by the method described in Malik and Singh (1980). For catalase activity, the permaganametric titration method was followed as shown by Kar and Mishra (1976).

3. Results and discussion

In *Z. mays*, 40 mM KNO_3 was found to be optimal for *in vivo* nitrate reductase activity in the leaves of different ages. Maximal nitrate reductase activity was observed only in the young leaves followed by moderately mature, mature and senescent leaves (figure 1). A similar observation was made in *V. unguiculata*, with
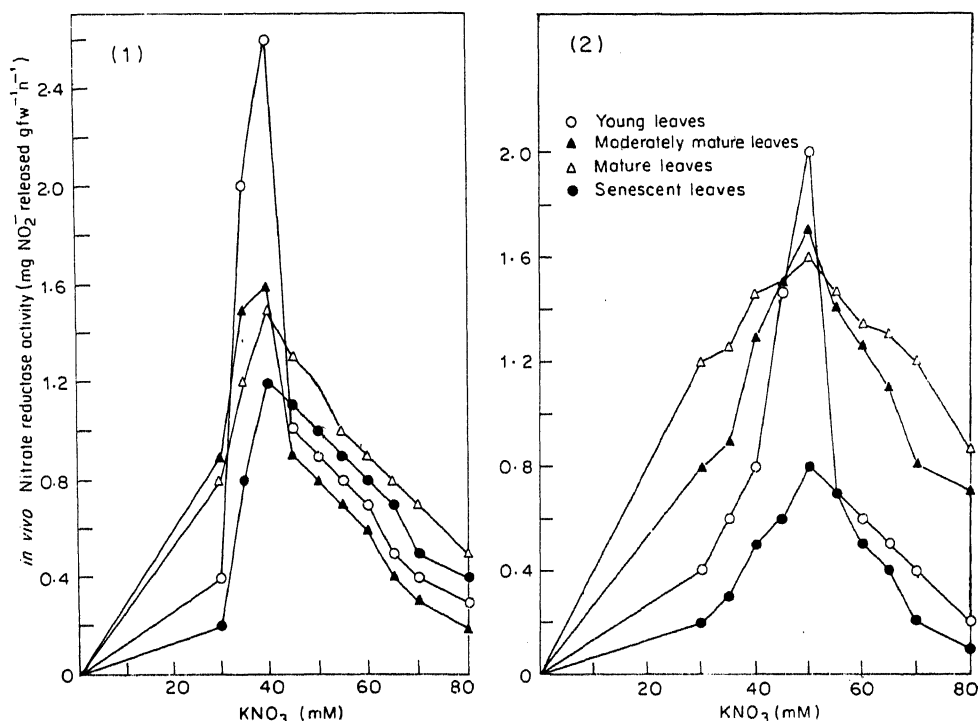
Figures 1 and 2. Optimisation of KNO_3 requirement for *in vivo* nitrate reductase activity in leaves of different ages of 30-day old light-grown plants of **(1)** *Z. mays* and *V. unguiculata*. The data are the average of two different experiments with duplicates.

50 mM KNO_3 as optimal requirement for nitrate reductase activity (figure 2). The two plants differed in their levels of nitrate reductase activity in the leaves of different kinds (table 1). The present observation of nitrate reductase activity in *Z. mays* is in agreement with the finding of Vivekanandan and Edwards (1987) that nitrate reductase activity is highest in the young leaves compared to mature leaf tissue.

Shifting of light-grown plants from light to dark resulted in considerable decline in the activity of nitrate reductase, confirming the well established link between nitrate reduction and photochemical reaction (Burstrom 1943). The present finding is supported by the work of Hisamatsu *et al* (1988) that light activated nitrate reductase in squash cotyledons decreased continuously in darkness. The results obtained through aminotriazole foliar spray (2·5 mM) on nitrate reductase activity is rather intriguing in that amitrole-sprayed leaves exhibited a strong inhibition of nitrate reductase activity in the young, the moderately mature leaves, whereas in mature leaves the inhibition was not significant and in senescing leaves no significant inhibition could be observed compared to the control (table 1). However, at 5 mM amitrole treatment considerable inhibition in nitrate reductase activity was observed. The severe inhibition of nitrate reductase activity in *Z. mays* and *V. unguiculata* leaves may be due to either complete or partial absence of chloroplasts which otherwise would have contained plastid-bound nitrate reductase.

Table 1. Effect of light and amitrole treatment on *in vivo* nitrate reductase peroxidase, polyphenol oxidase and catalase activities in 30-day old light-grown plants of *Z. mays* and *V. unguiculata.*

Enzyme activity	Plants	Leaf age	White	Dark 1 h	Dark 3 h	Amitrole 2·5 mM	Amitrole 5·0 mM
In vivo nitrate reductase (mg NO$_2^-$ released h^{-1} gfw^{-1})	*Z. mays*	Y	3·8	3·3	2·0	0·8	0·2
		MM	3·4	3·2	1·4	1·4	0·5
		M	3·1	3·0	1·2	2·1	0·9
		S	2·4	1·9	0·6	3·2	1·4
	V. unguiculata	Y	2·2	1·9	1·6	0·3	0·1
		MM	1·7	1·6	1·1	0·5	0·2
		M	1·5	1·4	0·7	1·2	0·4
		S	0·9	0·8	0·5	1·3	0·5
Peroxidase (OD unit min^{-1} gfw^{-1})	*Z. mays*	Y	8·0	6·4	4·0	53·0	72·0
		MM	21·6	12·8	6·4	21·6	57·6
		M	17·6	8·0	5·6	16·8	38·4
		S	10·4	7·2	4·8	11·2	10·4
	V. unguiculata	Y	8·8	13·6	16·0	42·0	93·0
		MM	11·2	18·4	31·2	38·0	86·0
		M	15·0	32·0	40·0	35·0	77·0
		S	32·8	53·6	70·4	19·0	70·0
Polyphenol oxidase (OD unit min^{-1} gfw^{-1})	*Z. mays*	Y	0·8	1·6	5·2	9·6	11·2
		MM	1·6	2·4	7·2	8·8	9·6
		M	2·8	3·6	9·6	7·6	8·0
		S	3·2	4·0	14·4	6·0	7·2
	V. unguiculata	Y	3·1	4·4	6·0	4·8	9·6
		MM	4·8	6·2	8·0	3·1	6·8
		M	5·6	7·2	9·0	2·0	5·6
		S	6·4	9·6	10·4	1·5	4·8
Catalase (μmol H$_2$O$_2$ released min^{-1})	*Z. mays*	Y	2·0	1·2	2·6	1·8	0·8
		MM	2·8	2·0	3·6	2·2	1·2
		M	4·8	2·8	5·6	3·6	2·4
		S	6·0	3·4	6·8	4·6	3·5
	V. unguiculata	Y	1·2	0·8	1·6	0·6	0·4
		MM	1·6	1·0	2·5	0·9	0·8
		M	2·0	1·6	4·4	1·2	0·9
		S	3·2	2·4	4·8	2·0	1·1

Y, Young; MM, moderately mature; M, mature; S, Senescence.

Amitrole-induced chlorotic leaves generally lacked chloroplasts but contained only proplastids (Vivekanandan and Gnanam 1974a).

In *Z. mays*, the activity of peroxidase increased upon maturity (ageing) of the leaves and declined at the time of senescence (table 1). In green plants kept in darkness for a period of 3 h, peroxidase activity was considerably reduced. This might point out that the enzyme activity is affected by light-dark transition

suggesting that the enzyme is light-activatable in green plants. This finding is supported by the increased peroxidase activity in light and its decrease in darkness in spinach and *Hydrilla* (Kar and Choudhuri 1987). Amitrole treatment considerably increased peroxidase activity in young, moderately mature and mature leaves and the level of increment depended on the kind of the leaves. There is evidence in literature that amitrole did not inhibit peroxidase activity (Sagisaka and Asada 1986). It may be visualized that peroxidase activity could be induced in amitrole-treated leaves (5 mM) by gradual induction of senescence as chloroplasts break down, and inhibition of chloroplast formation has been suggested to be the most striking feature of amitrole (Aaronson 1960; Bartels and Weier 1969; Vivekanandan and Gnanam 1975b).

The peroxidase activity in young and mature leaves of *V. unguiculata* was lower compared to the senescent leaves. Strangely enough, exposure of light-grown plants to darkness increased the activity of peroxidase significantly compared to the plants kept in continuous light. Amitrole (5 mM) caused substantial increase in the activity of the enzyme in young leaves (table 1). It is not clear from the present study why peroxidase activity should behave differently in *V. unguiculata* and *Z. mays*. The activity of peroxidase in *V. unguiculata* was always higher in senescing leaves and therefore, return of plants to dark might indicate an act of induction of senescence and hence higher activity of the enzyme.

The activity of polyphenol oxidase increased gradually from the young leaves up to maturity and senescence. This enzyme exhibited a tremendous increase in activity upon dark treatment. The increase in polyphenol oxidase activity was more in mature and senescent tissues as well as under dark treatment. The pattern of polyphenol oxidase activity induced by amitrole was quite different from that of the dark treatment in that the enzyme activity was maximally increased in the young leaves followed by moderately mature, mature and senescent leaves. The level of polyphenol activity was several times higher in *Z. mays* than what was observed in *V. unguiculata*. Amitrole treatment induced polyphenol oxidase activity more in young leaves than in mature leaves. However, the trend was reverse in untreated leaves. There is lot of evidence to support the view that polyphenol oxidase is located in chloroplast (Li and Bonner 1947; Tolbert 1973; Vaughn and Duke 1981, 1982). Although polyphenol oxidase is a chloroplast enzyme, it is not light-activatable as evidenced by the present study and this only indicates that not all chloroplast enzymes are activated by light although some of the calvin cycle enzymes are known to be light activated (Edwards *et al* 1985).

The activity of catalase increased from the young to senescent leaves, as was observed for polyphenol oxidase activity in *V. unguiculata* and *Z. mays*. Prolonged exposure to darkness (3 h) marginally increased the activity of catalase, whereas treatment with amitrole did not appreciably change the level of activity of catalase. Almost a similar pattern of enzyme activity was observed in *V. unguiculata* under dark as well as amitrole treatment. It may be mentioned that amitrole at 5 mM caused significant inhibition of catalase activity in *V. unguiculata*. Marginal to significant decrease in the activity of catalase exhibited by amitrole-treated leaves derives further support from the observations of Pyfrom *et al* (1957), Aaronson (1960) and Margoliash *et al* (1960). It is interesting to note that under amitrole treatment the activities of peroxidase and polyphenol oxidase and catalase showed inverse relationship in both *Z. mays* and *V. unguiculata* i.e., catalase activity

showing increment from the young to the senescing leaf tissue, while peroxidase and polyphenol oxidase activities showed a decline. Such an inverse relationship could not be observed in leaves of different ages of untreated control plants.

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Cytomixis in woody species

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Abstract. Cytomixis with actual transfer of chromatin material has been recorded in 6 woody species, from early prophase to telophase-II. It is more common at early stages of meiosis. Number of pollen mother cells involved vary from 2–8. As a consequence of chromatin migration both hypo and hyperploid meiocytes are seen in *Serissa foetida*, *Symplocos chinensis* and *Quercus semecarpifolia*. However, in *Cordia dichotoma* and *Salix elegans* lower and in *Caryopteris odorata* higher numbers than the normal complement are not countable due to stickiness or agglutination of chromosomes, respectively. Reduction in pollen fertility in these species is due to the cytomixis. The phenomenon is attributed to certain unknown genetic factors.

Keywords. Cytomixis; woody species; cytology.

1. Introduction

The first authentic report on the migration of chromatin material from one pollen mother cell (PMC) to another is by Gates (1911) in *Oenothera gigas* who named it as 'cytomixis'. Since then it has been described in a wide range of normal, hybrid and apomictic plants belonging to diverse families of angiosperms. The phenomenon had been more commonly known in herbaceous plants and was thought to be of sporadic and infrequent occurrence in the woody species, but the recent reports by Singhal and Gill (1985) and Chatha and Bir (1988) have shown that its rarity in woody taxa was perhaps due to the lack of intensive cytological work on them.

2. Materials and methods

For meiotic studies flower buds were fixed in Carnoy's solution. Anthers were squashed in 1% acetocarmine and observations pertaining to cytomictic connections were recorded in PMCs floating in a drop of stain, before placing coverglass, to avoid the possibility of distortion due to manual pressure. However, the detailed cytological observations were made on the well spread PMCs showing cytomixis at different stages. Slides were made permanent in euparal. Pollen fertility was estimated on the basis of their stainability with 1:1 glycero-acetocarmine and well filled nature.

3. Results

During the cytological studies on 200 woody species from Garhwal Himalaya and central India, the phenomenon of cytomixis is observed in 6 species (figures 1–13). Complete information on the cytomictic events recorded in these species is given in
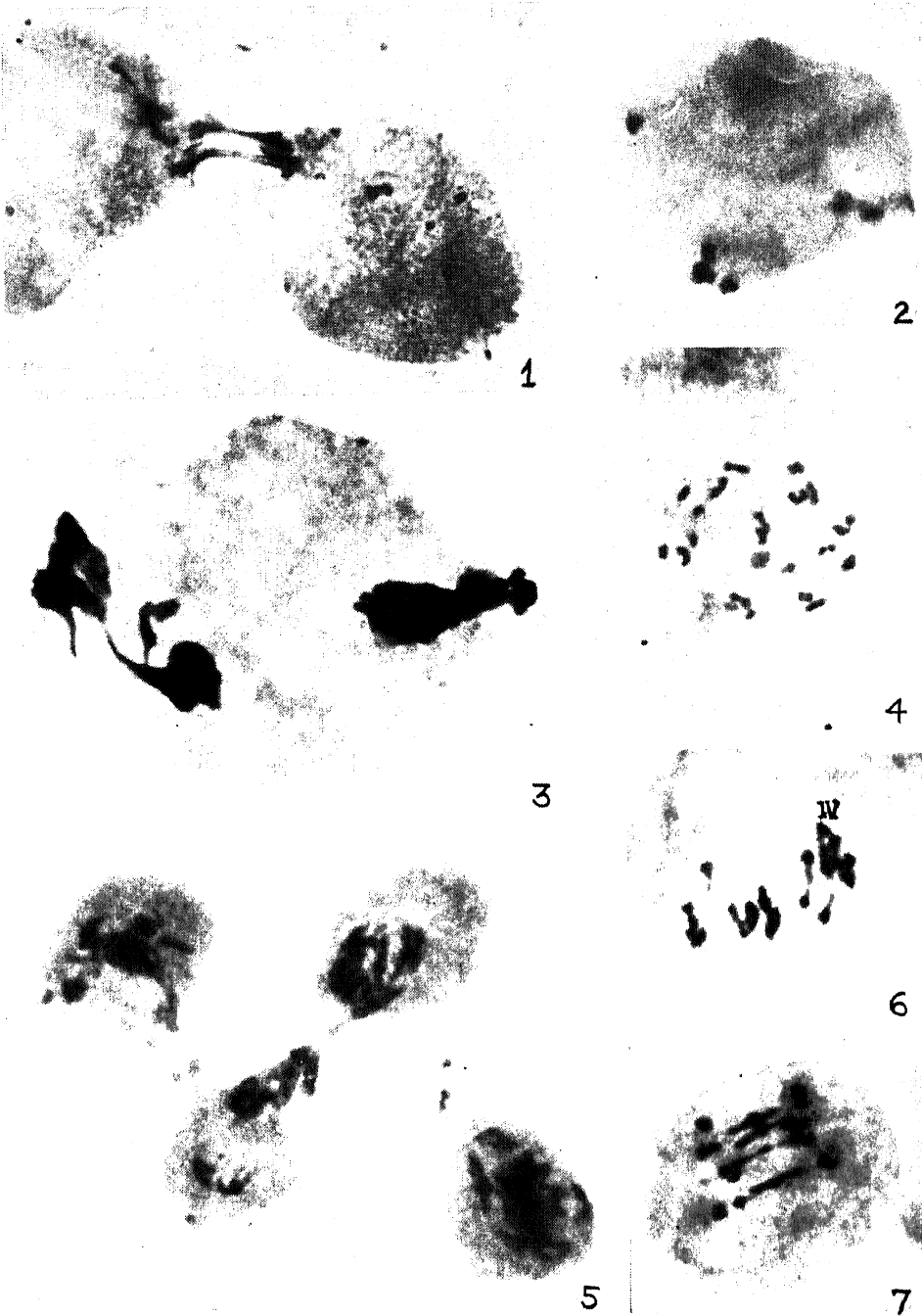
Figures 1–7. Cytomixis in PMCs (× 900). **1,2.** *Caryopteris odorata,* **1.** Two PMCs showing cytomixis. **2.** M–I with 6_{II}. **3.** *Cordia dichotoma,* PMCs involved in cytomixis. **4.** *Salix elegans,* M–I with $2n = 40 = 20_{II}$. **5–7.** *Symplocos chinensis.* **5.** Four PMCs showing chromatin transfer. **6.** M–I showing $1_{IV} + 9_{II}$. **7.** A–I with chromatin bridges.
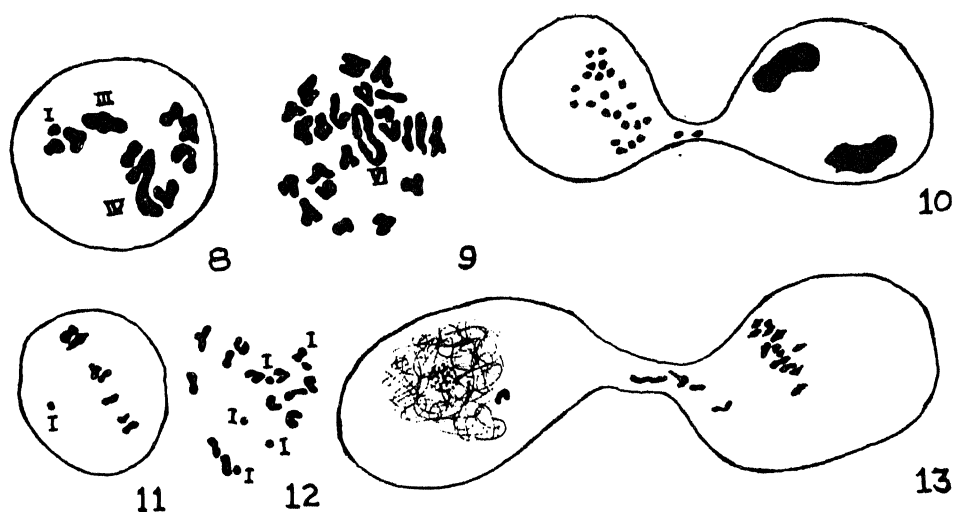
Figures 8–13. Cytomixis in PMCs ($\times 750$). **8.** *Caryopteris odorata*, M–I with $2n = 22 = 1_{IV} + 1_{III} + 7_{II} + 1_{I}$. **9.** *Cordia dichotoma*, M–I with $2n = 50 = 1_{VI} + 22_{II}$. **10–12.** *Quercus semecarpifolia*. **10.** PMCs at different stages showing cytomictic channel. **11.** M–I with $2n = 15 = 7_{II} + 1_{I}$. **12.** M–I with $2n = 31 = 13_{II} + 5_{I}$. **13.** *Salix elegans*, PMCs at different stages showing cytomixis.

Table 1. Data on cytomixis in the presently investigated species.

Taxon	2n chromosome number with ploidy level	PMCs* showing cytomixis (%)	PMCs showing actual transfer (%)	Number of PMCs involved	Meiotic stages of cytomixis	PMCs with 2n chromosome number	
						Lowest	Highest
Caryopteris odorata Robins	40 Tetraploid	23·69	100·0	2–3	Diakinesis and Metaphase I	12	40
Cordia dichotoma Forst. f.	48 Hexaploid	11·80	100·0	2–3	Metaphase I	48	50
Quercus semecarpifolia Sm.	24 Diploid	11·63	80·0	2	Early prophase to Anaphase I	15	32
Salix elegans Wall.	38 Diploid	12·00	35·0	2	Early prophase to Metaphase I	38	40
Serissa foetida Lam.	22 Diploid	23·90	100·0	2–8	Early prophase	18	24
Symplocos chinensis Druce	22 Diploid	16·25	28·5	2–4	Early prophase Telophase II	16	26

*Analysis based on atleast 80 PMCs.

table 1, while observations on the species showing other points of cytological significance are given below.

3.1 *Symplocos chinensis*

In one PMC at M–I, configuration of $2n = 22 = 1_{IV} + 9_{II}$ (figure 6) has been seen. At

anaphase-I, in 14·3% of the observed PMCs, 1–4 chromatin bridges (figure 7) are recorded.

3.2 *Cordia dichotoma*

An individual tree with cytomixis also shows some multiple associations in 73·9% of PMCs at diakinesis/M–I (table 2) while in rest of the PMCs, 24 bivalents are regularly constituted. Separation at anaphases is normal. Due to clumping of chromosomes, number lower than $2n=48$ could not be counted while an increase in number is evident from one PMC at M–I showing $2n=50=1_{VI}+22_{II}$ (figure 9).

3.3 *Caryopteris odorata*

Cytomixis results into both hypo and hyperploid cells, accordingly at diakinesis/M–I, PMCs with $2n=12=6_{II}$ (figure 2) and $2n=22=1_{IV}+1_{III}+7_{II}+1_{I}$ (figure 8) are observed. Laggards at anaphases lead to abnormal microsporogenesis, where besides normal tetrads (68·5%), dyads (11·8%), triads (8·7%) and polyads (11%) are seen. Pollen fertility is reduced to 87·5%.

4. Discussion

From table 3, it is evident that, todate, the phenomenon of cytomix is recorded in 51 woody genera (56 species). The percentage of cells connected with cytomictic channels vary from 11·63% (*Quercus semecarpifolia*) to 23·90% (*Serissa foetida*) and all the species show actual transference of chromatin material, though at variable rates (table 1).

The nondirectional movement of chromatin material in presently studied species is in line with the observations of Chatha and Bir (1988) in different plants, while Gates (1911) has shown that in *Oenothera gigas* the migration of chromatin material is always unidirectional. In *Serissa foetida* and *Salix elegans* the movement of nucleolus has been associated with the transfer of chromatin material like *Papaver rhoeas* (Chauhan 1981).

Cytomixis occurs at all stages of meiosis i.e. early prophase to telophase-II.

Table 2. Chromosomal associations in *Cordia dichotoma*

PMCs observed		Configurations		
Number	Per centage	2 IV	IV	II
15	65·2	—	1	22
6	26·1	—	—	24
2	8·7	1	—	20
Total 23	100·0	2	15	514
Average frequency/PMC		0·09	0·65	22·35
Percentage of chromosomes involved		1·3	5·6	93·1

Table 3. Incidence of cytomixis in woody genera.

Investigator(s)	Genera
Woodworth (1929)	*Alnus* and *Corylus*
Youngman (1931)	*Thespesia*
Smith-White (1948)	*Callistemon*
Salesses (1970)	*Prunus*
Mehra (1972)	*Casearia, Cornus, Symplocos, Citharexylum* and *Castanopsis*
Krishnan (1980)	*Aphloia, Casearia, Cochlospermum, Flacourtia* and *Xylosma*
Srivastav and Raina (1980)	*Clitoria*
De and Sharma (1983)	*Ervatamia*
Singhal and Gill (1985)	*Caragana, Casearia, Cocculus, Cotoneaster, Dodonaea, Eugenia, Flacourtia, Glycosmis, Helicteres, Hiptage, Hydoncarpus, Hydrangea, Philadelphus, Prunus, Semecarpus, Sorbus* and *Stigmaphyllon*
Chatha and Bir (1988)	*Gmelina, Hippophae, Jasminum, Lantana, Ligustrum, Lonicera, Olea, Pavetta, Psychotria, Symphorema, Symplocos, Tabernaemontana, Vaccinium, Viburnum, Wikstroemia* and *Wrightia*
Banerjee and Sharma (1988)	*Rauwolfia*
Present studies	*Caryopteris, Cordia, Quercus, Salix, Serissa* and *Symplocos*

However it is more frequent at early prophase (Sarvella 1958; Chauhan 1981; Singhal and Gill 1985; Chatha and Bir 1988). Its existence even at tetrad stage has been recorded in *Ervatamia divaricata* by De and Sharma (1983). In the present study, in different species, cytomixis is noticed to occur at different stages of meiosis (cf. table 1). Usually cytomixis takes place when the participating cells are at the same stage of division, however, in *Gossypium* (Sarvella 1958) and *Justicia transquobariensis* (Saggoo and Bir 1983) cytomictic connections between PMC at different stages of meiosis are on record. Presently also such a phenomenon has been seen in *Q. semecarpifolia* and *S. elegans* where connections between PMCs at A–I and T–I (figure 10) and early prophase and M–I (figure 13) are seen, respectively.

Cytomixis has caused deviation in chromosome number both on the positive and negative sides of the normal number (see table 1). As pointed out earlier by Omara (1976) and Chauhan (1981), in the hyperploid cells of all the presently studied individuals (except *Q. semecarpifolia* and *Cordia dichotoma*) normal bivalent formation is seen. It is indicative of the fact that in these plants migration of chromatin material has taken place after the initiation of chromosome pairing. This contention is further supported by the fact that in these plants the deviating cells show even number of chromosomes which indicate that the chromosomes have migrated in pairs. In *C. dichotoma*, a hyperploid PMC with $2n = 50 = 1_{VI} + 22_{II}$ (figure 9) is seen. Since the plant is showing structural hybridity for up to 8 chromosomes, it is difficult to pin point that this hexavalent is the result of translocation heterozygosity or due to the migration of chromatin before the initiation of chromosome pairing. In *Q. semecarpifolia* both hypo- and hyperploid cells show bivalents and univalents. In certain PMCs odd chromosome numbers

such as 2n = 15, 31 (figures 11, 12) are seen which indicate that cytomixis has occurred before the onset of chromosome pairing.

There are conflicting views and explantations about the cause and significance of cytomixis in plants as it is both considered to be an artifact or naturally occurring phenomena by different workers (Singhal and Gill 1985). In all the presently studied species (excluding *C. dichotoma*), since normal bivalent formation is seen at diakinesis/M–I, the phenomenon seems to be natural, under some genetic control as has been suggested by Brown and Bertke (1974) and Omara (1976). In *C. dichotoma*, its occurrence might be due to meiotic abnormalities (Stebbins 1958) because the taxon is polyploid with structural heterozygosity for as many as 8 chromosomes.

Acknowledgements

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Competitive fitness of *Centella asiatica* populations raised from stem cuttings and seedlings

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Abstract. *Centella asiatica* (Linn.) Urb., a clonal perennial herb, grows abundantly on a wide range of habitats in Meghalaya and reproduces both through vegetative and sexual means. The paper presents the competitive interaction between the populations of *Centella asiatica* raised from stem cuttings and seedlings, designated as 'C_c' and 'C_s' respectively. The two categories of plants showed significant differences in growth performance. The numbers of stolons and seeds produced by 'C_c' were greater than by 'C_s' in both monoculture and mixtures. The total leaf area and dry matter yield of 'C_c' were greater in monoculture than in mixtures, while the reverse was true with 'C_s'. A comparison of the two categories of plants in monoculture and mixtures reveals that with increased proportion (75%) of C_s in mixture, the yield of C_c increased while the yield of C_s decreased in mixed populations having 75% C_c, thus depicting the competitive superiority of C_c over C_s. The relative yield ratio of C_c to C_s which was greater than unity also confirms that population of *Centella asiatica* raised from the stem cuttings is more competitive than that developing from the seedlings.

Keywords. Competitive fitness; *Centella asiatica*; perennial herb; monoculture.

1. Introduction

Many perennial herbs rely exclusively on clonal multiplication once they have colonized a site and the seedlings if present, rarely survive to adulthood (Sarukhan and Harper 1973; Turkington *et al* 1979; Schmid 1984; Hartnett and Bazzaz 1985). In these plants the genetic changes within a population are largely due to the changes in the abundance and distribution of genets which get established during the initial colonization phase (Schmid 1985). The con-specific populations have been reported to differ considerably in their competitiveness (Snaydon 1971; Ford 1981; Bazzaz *et al* 1982; Heywood and Levin 1984; Clay and Levin 1986; Lee *et al* 1986). Studies on competition between the populations of *Agropyron repens* and *A. canicum* raised from seeds and from tillers (Tripathi and Harper 1973) and between populations of *Imperata cylindrica* raised from seeds and rhizomes (Kushwaha *et al* 1983) reveal that competitive success of plants also depends on the propagules from which they are produced. Such a study may add to our understanding of natural co-existence of genets and ramets and their ultimate contribution to the population maintenance of those species which reproduce both sexually as well as asexually.

   Centella asiatica (Linn.) Urb. (Apiaceae), a clonal herbaceous perennial is among the most important medicinal plants and in Meghalaya it is used for curing stomachache, dysentery and as blood purifier (Wankhar and Tripathi 1987). The plant is widely spread in Meghalaya under a variety of ecological conditions. It reproduces both through vegetative and sexual means, although the latter mode of

reproduction is negligible. Local patch expansion occurs primarily as a result of clonal growth. In nature the seedling survival is very low (Wankhar 1987). In field conditions, however, populations originating from the seedlings and from the vegetative propagules do come in contact with each other and compete for the available resources. The success of genets depends in large measure, upon the 'stress' created by its own asexually produced allies. An analysis of relative growth of the populations raised from seeds and from stem cuttings in pure and mixed stands may prove rewarding in understanding the population biology and life cycle strategies of such perennial species.

2.   Materials and methods

The experiment was performed in a polythene-covered net house. The stem cuttings of uniform length (3·5–4·0 cm, bearing a node) and weight (20–30 mg) and seedlings having 2–3 leaves (10–15 mg dry weight) were collected from the natural population. Keeping the overall density constant (4 plants/pot), the stem cuttings and seedlings were grown in the pots (21 cm diameter, 19 cm depth with a basal drainage hole) filled with garden soil in the ratios of 100:0, 75:25, 50:50, 25:75 and 0:100, so as to give a 'replacement series' (De Wit 1960). The experiment consisted of 5 types of stands × 3 harvests × 5 replicates; thus involving in all 75 pots. The pure and mixed populations of individuals raised from the stem cuttings and seedlings were maintained in the pots with the following density combinations:

(i)   Pure population raised from the stem cutting (4 plants/pot).
(ii) Three plants from the stem cutting + 1 plant from the seedling.
(iii) Two plants from the stem cutting + 2 plants from the seedling.
(iv) One plant from the stem cutting + 3 plants from the seedling.
(v)   Pure population raised from the seedlings (4 plants/pot).

The planting densities of the stem cuttings and seedlings were 3 times that of the population density of each type of individuals desired to be maintained in the experimental pots. After the cuttings sprouted and the seedlings established, the population was thinned down to the desired density of 4 plants per pot for both pure and mixed stands.

The experiment was started on September 4, 1985 and terminated on July 10, 1986. The 3 harvest were taken at 3 months interval after planting. At each harvest, stolon production, leaf area and dry matter yield were determined. Relative yield (RY), relative yield ratio (RYR) and relative yield total (RYT) (De Wit and Van den Bergh 1965) were computed from the yield data. For the sake of convenience, plants raised from the stem cuttings and seedlings have been designated as 'C_c' and 'C_s' respectively. The data were statistically analysed using ANOVA. The SE of means are given wherever necessary.

3.   Results

The two categories of plants showed significant differences in growth performance. After 3 months growth, no stolon was produced either by 'C_c' or 'C_s', however, after 6 months growth a few stolons were produced. The production of stolons by 'C_c'

and 'C$_s$' was almost similar when they were grown in pure or mixtures. In general the number of stolons produced by 'C$_c$' was greater than by 'C$_s$' (table 1).

The total leaf area and the total yield per pot in mixture were intermediate between the pure stands of both C$_c$ and C$_s$ (figures 1, 2). Seed output of plants

Table 1. Number of stolons and seeds produced per plant in pure and mixed populations raised from cuttings and seedlings of *C. asiatica* at the final harvest (SEs given along with means).

Nature of stands	Stolons	Seeds
C. asiatica raised from stem cuttings (C$_c$)		
Pure stand (100%)	6·6 ± 0·14	61·9 ± 5·4
Mixed stands		
C$_c$ (75%) grown with C$_s$ (25%)	6·7 ± 0·28	62·3 ± 0·7
C$_c$ (50%) grown with C$_s$ (50%)	6·4 ± 0·19	61·4 ± 0·3
C$_c$ (25%) grown with C$_s$ (75%)	7·0 ± 1·9	67·2 ± 0·5
C. asiatica raised from seedlings (C$_s$)		
Pure stand (100%)	4·4 ± 0·38	26·4 ± 0·9
Mixed stands		
C$_s$ (75%) grown with C$_c$ (25%)	4·8 ± 0·28	29·7 ± 0·49
C$_s$ (50%) grown with C$_c$ (50%)	4·5 ± 0·24	27·9 ± 1·65
C$_s$ (25%) grown with C$_c$ (75%)	4·2 ± 0·47	25·2 ± 1·42
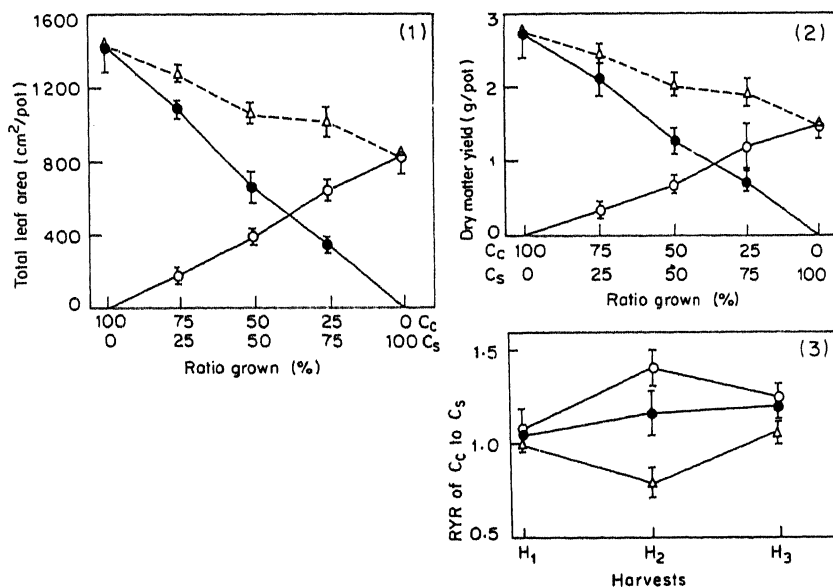
Figures 1–3. 1. Total leaf area/pot (cm^2) in pure and mixed populations of *C. asiatica* raised from the stem cuttings and seedlings after 9 months growth. Leaf area in pure population raised from the (●) stem cuttings (C$_c$), (○) seedlings (C$_s$) and (△) mixed populations. **2.** Dry matter yield in pure and mixed populations of *C. asiatica* raised from the stem cuttings and seedlings after 9 months growth. Yield in pure population raised from the (●) stem cuttings, (○) seedlings and (△) mixture. **3.** Relative yield ratio of the population raised from stem cuttings (C$_c$) to that from seedlings (C$_s$) after 9 months growth. (○), 75% C$_c$ + 25% C$_s$; (●), 50% C$_c$ + 50% C$_s$; (△), 25% C$_c$ + 75% C$_s$. Vertical lines attached to symbols show SD.

developing from the cuttings was more compared with that of plants raised from the seedlings (table 1).

Relative yield of 'C_c' was significantly ($P < 0.05$) greater than that of 'C_s' (table 2). The RYR values were always greater than one (figure 3), which indicates that 'C_c' is more competitive than 'C_s'. The RYT worked out to be more than one at all harvests (table 3).

4. Discussion

The observed differences between the growth of plants raised from the stem cuttings and seedlings could be related to differences in initial weight of the propagules as reported by Tripathi and Harper (1973) and Kushwaha *et al* (1983) in other perennial plants. The large differences in survival and growth between seedlings and transplants of *Trifolium repens* observed by Turkington *et al* (1979) were also attributed to the initial differences in plant size between seedlings and ramets. Abrahamson (1980) argued that where both vegetative and sexual reproduction occur simultaneously, the vegetative offspring will develop immediately and quickly

Table 2. Relative yield of *C. asiatica* raised from stem cuttings and seedlings at the 3 harvests.

Nature of stand	Relative yield		
	H_1	H_2	H_3
C. asiatica raised from stem cuttings (C_c)			
C_c (75%) grown with C_s (25%)	0.97	1.06	1.07
C_c (50%) grown with C_s (50%)	0.98	1.04	1.14
C_c (25%) grown with C_s (75%)	0.97	0.89	1.13
C. asiatica raised from seedlings (C_s)			
C_s (75%) grown with C_c (25%)	0.96	1.12	1.06
C_s (50%) grown with C_c (50%)	0.92	0.89	0.95
C_s (25%) grown with C_c (75%)	0.90	0.76	0.86
Sources of variation	Probability		
Nature of stand	<0.01		
Harvest	<0.01		
Interaction	<0.05		

Table 3. Relative yield total of *C. asiatica* in different mixed populations at the 3 harvests.

Nature of stand	Relative yield total		
	H_1	H_2	H_3
C_c (75%) grown with C_s (25%)	1.87	1.82	1.93
C_c (50%) grown with C_s (50%)	1.90	1.93	2.03
C_c (25%) grown with C_s (75%)	1.93	2.01	2.19
Sources of variation	Probability		
Nature of stand	<0.05		
Harvest	<0.05		

become an adult due to larger food supply in the initial stage of growth. Zangerl and Bazzaz (1983) reported that larger food reserves in the rhizomes of *Polygonum* compared to its seeds permit the plants of rhizomatous origin to persist in resource-limited environments where plants derived from seeds do not succeed.

There were clear growth advantages gained by the plants raised from stem cuttings at the initial stages but at the later stages the differences had narrowed down so much so that the growth and production of stolons in plants developing from the seedlings and stem cuttings were more or less equal. Keeping in view the low density populations raised in the pots, it could be argued that if ample space is available the seedlings would not suffer extreme growth suppression and may successfully grow into adult plants in nature. As reported by various workers (e.g. Sagar and Harper 1960; Cavers and Harper 1967; Putwain and Harper 1970; Rai and Tripathi 1985), many other species also show little establishment and survival in closed vegetation.

The greater RY and RYR values for C_c compared with C_s depict that the population raised from the stem cuttings is more competitive than that from the seedlings. This agrees with the findings of Tripathi and Harper (1973) and Kushwaha *et al* (1983). The RYT values (RYT > 1) obtained in the present study suggest facilitation as reported by Clay and Levin (1986) and indicates that the two populations can co-exist provided that there was no crowding. The density per pot was kept quite low in the present study keeping in view that the plant produces stolons which have potentiality to root at each node. This ensured that the pots do not quickly get depleted of resources. The replacement series competition experiments suffer from several limitations as discussed by Taylor and Aarssen (1989) but they definitely provide a sensitive technique by which to compare the competitive behaviour of the species in pure and mixed populations. Taylor and Aarssen (1989) maintain that competitive abilities of the species are density–dependent making it difficult to choose an appropriate density to conduct an experiment. This sensitivity to density, as argued by Firbank and Watkinson (1985) represents a weakness of the replacement-series design in not providing a consistent measure of competitive abilities across a range of densities. In a plant like *C. asiatica* where density increased with the passage of time, the remarkable consistency with which RY values of populations raised from the stem cuttings and seedlings differed at the 3 harvests (table 2) suggests that the problem of choosing an appropriate density for conducting replacement-series experiment does not pose serious difficulty except that care should be taken to choose a density which provides for future population expansion through vegetative means during experimental duration.

Sexual reproduction in *C. asiatica* is rendered to be of little ecological significance due to the low production of seeds (table 1) and small soil seed bank ($2208/m^2$) and considerable loss of seed viability (10%) on burial in natural conditions (Wankhar 1987). The small number of seedlings of *C. asiatica* that appeared in nature may be the result of not only a small seed bank, but of the unmeasured effects of competition from the established plants of *C. asiatica* and other species which grew in their close proximity. There is close parallel between this plant and *Agrostis stolonifera* which also has a small seed bank in soil and produces seedlings that are less competitive (Howe and Chancellor 1984). Grime (1979) suggested that the ability of *A. stolonifera* to dominate the older swards is largely due to its ability to

spread vegetatively and the same holds true for *C. asiatica*. As pointed out by Alpert and Mooney (1980) and Hartnett and Bazzaz (1983), the risks involved in changing from heterotrophic nutrition to autotrophic life make this stage hazardous, while the daughter rosettes which develop with a continuous supply of resources from the parent plants are able to tolerate the dense situations. The competitive superiority of the population raised from the stem cuttings over that from the seedlings and predominant role of vegetative means of reproduction as reflected by the production of daughter rosettes in large numbers (Wankhar 1987), seem to constitute a viable ecological strategy of this plant enabling it to thrive successfully even in competitive sward situations.

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Some interesting Gasteromycetous fungi from eastern Himalaya

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Abstract. Eight species of Lycoperdales have been described. Five of them are recorded here for the first time from India and Himalaya. Genus *Morganella* is reported for the first time from India.

1. Introduction

Fungal forays to various localities in eastern Himalaya during 1977–81 including the Royal Kingdom of Bhutan, yielded several interesting species of Lycoperdales (Gasteromycetes). This paper provides detailed account of 8 such species, viz. *Vascellum endotephrum*, *V. curtisii*, *Morganella subincarnata*, *Bovista fusca*, *B. aspera*, *B. trachyspora*, *B. aestivalis* and *B. coprophila*. Except for *Vascellum curtisii*, *Bovista fusca* and *B. trachyspora*, the remaining 5 species and the genus *Morganella* are new records for India. All the collections have been deposited in the Herbarium, Department of Botany, Panjab University, Chandigarh (PAN) and in other herbaria mentioned against each collection number.

2. Observations

2.1 *Vascellum endotephrum* (Pat.) Demoulin and Dring, Bull. Jard. Bot. Nat. Belg., **45**: p. 358, 1975.
Syn: *Lycoperdon endotephrum* Pat., Bull. Soc. Mycol. Franc, **18**: 300, 1902. Figures 1 and 9.

Fructifications gregarious, scattered, globose, sub-globose or depressed globose, usually broader than tall, plicating below or with a thick, short stem-like base, up to 2·5 cm in diameter. Exoperidium yellowish brown or brown, spinose, spines brown, singly or in groups, usually convergent at the tips, more prominent in the upper part than towards the base, falling away at maturity. Endoperidium pale yellow to greyish yellow or light brown, membranous, conspicuously pitted, dehiscing by an apical torn stoma. Subgleba pale brown, moderately developed, occupying the short, thick stem-like base, chambered, chambers distinct, separated from the gleba by a prominent, membranous diaphragm. Gleba olive brown to brown, pulverulent. Paracapillitium threads hyaline, sparsely branched, septate, septa at regular intervals, encrusted with the glebal membranes debris, up to 9 μm wide. Basidiospores subglobose to ovoid, sometimes globose, 3·8–5·5 × 2·8–4·8 μm,

light brown, verruculose, verrucae distinct, cyanophilous, projecting into a thin hyaline envelope, guttulate, usually with a stump of pedical.

Collections examined (6 collections): Meghalaya: Khasi hills, Shillong, Happy valley, on road side, BMS 23054 (PAN), June 26, 1978.

   V. endotephrum is a tropical species and appears to be fairly distributed in the eastern Himalaya. It is characterised by brown spines of the exoperidium and conspicuously pitted endoperidium. It is recorded for the first time from India.

2.2   *Vascellum curtisii* (Berk.) Kreisel, Feddes Report, **68**: 86, 1963.
Syn: *Lycoperdon curtisii* Ber., Grevillea 2: 50, 1873.
L. wrightii Berk. and Curt., Grevillea 2: 50, 1873. Figures 2 and 10.

Fructifications caespitose, gregarious, scattered, sub-globose or depressed globose, usually broader than tall, plicating below to the point of attachment or with a short, thick stem-like base, up to 2·5 cm in diameter. Exoperidium orange white or white when young and fresh, turning to yellowish at maturity, spinose, spines conical, in stellate groups, with their tips cohering, intermixed with granular material, prominent in the upper part becoming smaller towards the base, falling away in patches at maturity in the upper part. Endoperidium yellowish grey to brownish grey, membranous, inconspicuously pitted, dehiscing by an apical torn stoma. Subgleba moderately developed, well-developed in some specimens, light brown or olive brown, chambered, chambers distinct, separated from the gleba by a thin membranous diaphragm. Gleba olive brown to brown, pulverulent. Paracapillitium threads hyaline or subhyaline, branched, septate, thin-walled, encrusted with glebal membranes debris, up to 5·5 μm wide. Basidiospores globose to subglobose or ovoid, 3·5–4·5 μm in diameter, pale brown, verruculose, verrucae cyanophilous, projecting into a thin, hyaline envelope, usually with a short stump of pedicel, guttulate.

Collection examined: Bhutan, Thimphu; Bunakha, on grassy ground, BMS 23366 (PAN), July 29, 1981.

Remarks: Ahmad (1942) first described this species from India as *Lycoperdon wrightii* Berk. and Curt., based on his collection made from Mussoorie hills (western Himalaya). Bowerman (1961) regarded *L. wrightii* as synonym of *L. curtisii*, which was later transferred to the genus *Vascellum* as *V. curtisii* by Kreisel (1963). Bhutan collection is quite typical of the species as it matches very closely with the descriptions by Kreisel (1963) and Ponce de Leon (1970).

2.3   *Morganella subincarnata* (Peck) Kreisel and Dring, Feddes Report, **74**: 117, 1967.
Syn.: *Lycoperdon subincarnatum* Peck., Ann. Rep. New York State Mus. Nat. Hist., **24**: 1872. Figures 3 and 11.

Fructifications gregarious, scattered, depressed globose, usually broader than tall, narrowing below into a short, thick base, up to 1·8 cm in diameter. Exoperidium brown to greyish brown above, pale yellow or pinkish below, spinulose, spines dark brown, more prominent in the upper part, becoming smaller and scattered below,

falling away at maturity. Endoperidium greyish yellow or pale brown, thin and membranous in the upper part, becoming gradually thickened towards the base, marked all over by small pits, outlined by ridges, giving the surface reticulate appearance, dehiscing by an irregular apical stoma. Subgleba moderately to well-developed, occupying the short stem-like base, chambered, chambers distinct. Gleba olive brown or brown, pulverulent. Glebal membranes abundant, hyaline, corrugated, cyanophilous. Paracapillitium threads hyaline, unbranched, septate, thin-walled, cyanophilous, encrusted with glebal membrances debris, up to 5·5 μm wide. Basidiospores globose, 3·5–7·0 $(-8·5)\mu$m in diameter, verruculose to echinulate, verrucae or spines projecting into a thin, hyaline envelope, usually with a short stump of pedicel.

Collections examined: Meghalaya: Khasi hills, Cherrapunji, on dead decaying angiospermous log, angiospermous forest, BMS 23077 (PAN), July 18, 1978.

Remarks: *Morganella subincarnata* was uptil now known only from North-America. The species can easily be distinguished by its pitted endoperidium. It is close to *M. compacta* (Cunn.) Kreisel and Dring, but can be separated by the larger spines of the exoperidium and pitted endoperidium.

Eastern Himalayan collection agree closely with the description of *M. subincarnata*, as given by Kreisel and Dring (1967).

2.4   *Bovista fusca* Lev., Ann. Sci. Nat., **5**: 303, 1846. Figures 4 and 12.

Collections examined (27 collections): West Bengal, Darjeeling, Algarah, on moist soil, *Cryptomeria japonica* forest BMS 23032 (PAN, LG), October 27, 1977.

Remarks: This species is very widely distributed in the temperate forests of the eastern Himalaya. It was previously recorded from India as *Bovista bovistoides* (Cke. and Mass.) Ahmad and is very common in the western Himalaya. Our collections are marked by globose to subglobose, sessile fructifications (up to 4 cm in diameter); granular or spinulose exoperidium which separates from the endoperidium or sometimes persisting as small mealy squamules; greyish brown to brown or dark brown, smooth, membranous endoperidium; absence of subgleba; olive brown to brown or dark brown gleba; '*Bovista*' type capillitium, i.e. of discrete units, consisting of olive to brown or dark brown main stem and lighter tapering branches usually aseptate, sometimes septate, thick-walled (wall up to 5 μm thick), unpitted, up to 16 $(-21)\mu$m wide; globose or subglobose to ovoid (4·5–6·5 ×'4·5 μm), verruculose and long pedicellate (pedicel up to 12 μm long) basidiospores.

It is difficult to distinguish between *B. fusca* Lev. and *B. bovistoides* (Cke. Mass.) Ahmad, due to the greater intraspecific variations. These two species are generally separated on the basis of the colour of the endoperidium and shape of spores and size of fructifications. All these features have been found to be quite variable even in the specimens of a single collections. Dr. Demoulin (Belgium), to whom some of the eastern Himalayan collections were referred to comments expressed similar views and remarked that "I do not belive any more *B. fusca*, *B. fulva* and *B. bovistoides* are distinct and lump them under the earliest name *B. fusca*". We have also found it to be very difficult to separate *B. fusca* from *B. bovistoides*, as none of the features is constant by which the two species can be distinguished. *B. bovistoides* is considered here as a synonym of *B. fusca* which is an earliest name of the fungus.
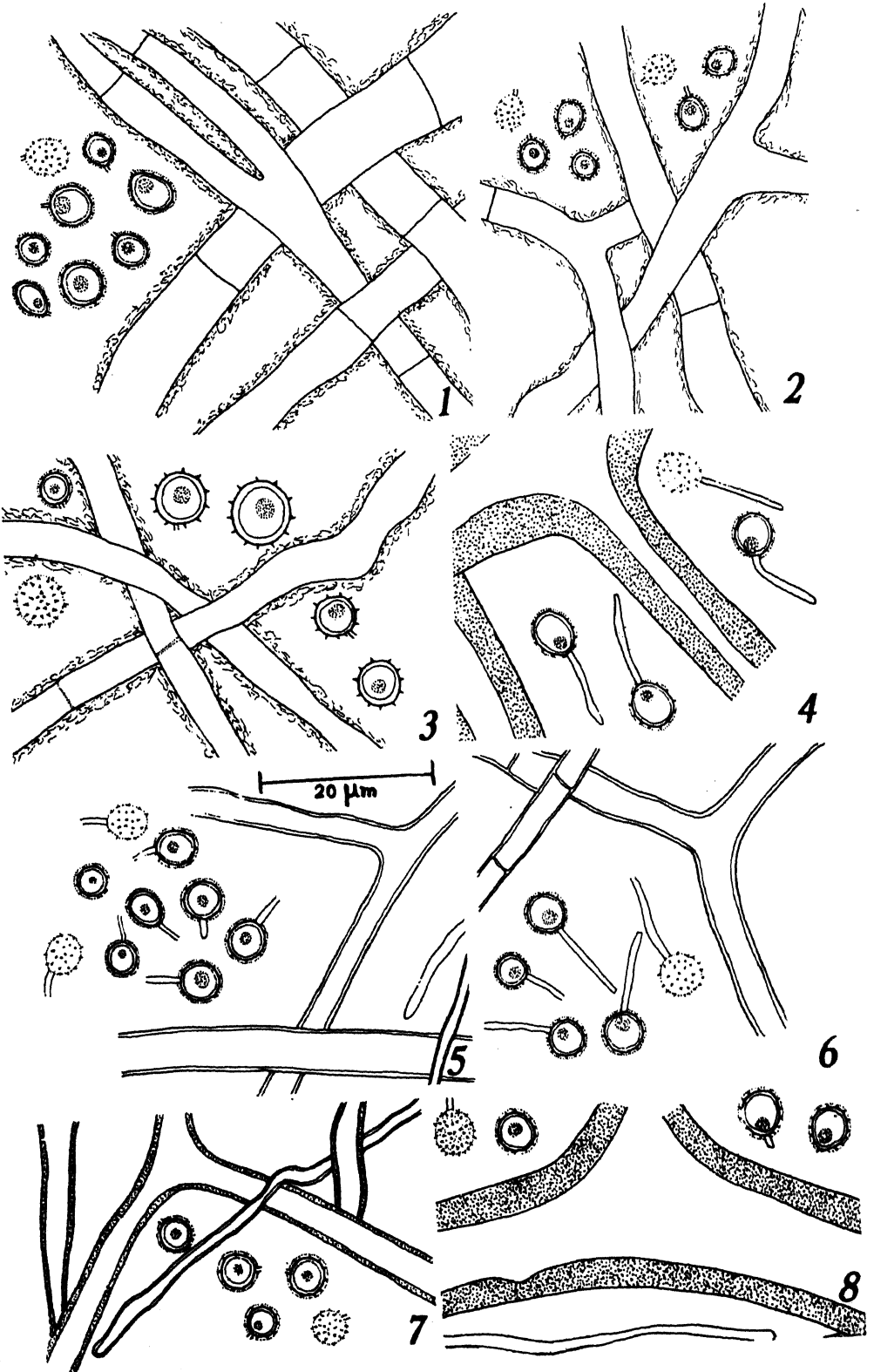
Figures 1–8.

2.5   *Bovista aspera*   Lev., Ann. Sci. Nat. Ser. 3 (Bot.) **5**: 162, 1846. Figures 5 and 13.

Fructifications scattered singly, subglobose, with a prolonged narrow base, up to 3·5 cm in diameter. Exoperidium creamish white when young and fresh, changing to light brown to brown at maturity, furfuraceous or spinulose, spines dense and prominent in the upper part becoming smaller and scattered towards the base, falling away at maturity beginning from the stoma. Endoperidium yellowish grey or light brown, smooth, shining, membranous, dehiscing by an irregular apical stoma. Subgleba light brown, compact, reduced. Gleba olive brown and pulverulent at maturity. Capillitium threads '*Lycoperdon*' type, olivaceous yellow, apices narrowly acuminate and lighter coloured, branched, aseptate but sometimes septate near the apices, thin-walled, wall up to 1 μm thick, pitted, pores few, up to 6 μm wide. Basidiospores globose or subglobose, 4·2–5·5 μm in diameter, olive yellow, verruculose, verrucae visible when stained in cotton blue, projecting into a thin, hyaline envelope, guttulate, pedicellate, pedicel short, up to 6 μm long.

Collections examined: West Bengal (3 collections): Darjeeling, on way to Sukhia, road side, BMS 23137 (PAN, LG), August 21, 1979.

Remarks: This species has been collected for the first time outside its type locality, Chile. Reduced, compact subgleba, pitted capillitial threads and globose or sub-globose basidiospores are the chief features of this species. PAN collections agree very well with the description of *B. aspera* as given by Kreisel (1967) except for short pedicels on spores (5·5–15·5 μm long pedicels reported for *B. aspera* by Kreisel 1967).

2.6   *Bovista trachyspora*   (Lloyd) Kreisel, Feddes Report **69**: 202, 1964. Figures 6 and 14.
Syn: *Bovistella trachyspora* Lloyd, Myc. Notes 2: 287, 1906.

Fructifications gregarious, scattered, singly, globose, or subglobose, up to 1·2 cm in diameter, creamish white when young and fresh, changing to greyish or dull yellow and finally to yellowish brown or brown at maturity. Exoperidium comprises a thin coat of minute spines, which are equally distributed throughout, intermixed with granular material, falling away at maturity; endoperidium yellowish grey to brown or dark brown, membranous, dehiscing by a small apical stoma. Subgleba absent. Gleba olive brown, pulverulent. Capillitium threads 'Lycoperdon' type, olivaceous brown to brown, lighter toward the apices, dichotomously, loosely branched, unpitted, frequently septate, thin-walled, wall up to 1 μm thick, up to 5 μm wide. Basidiospores globose, 3·5–5 μm in diameter, brown, verruculose, verrucae distinct in cotton blue, projecting into a thin, hyaline envelope, pedicellate pedicel hyaline, up to 10 μm long, guttulate.

Figures 1–8. Basidiospores and Capillitium. **1.** *V. endotephrum.* **2.** *V. curtisii.* **3.** *M. subincarnata.* **4.** *B. fusca.* **5.** *B. aspera.* **6.** *B. trachyspora* **7.** *B. aestivalis.* **8.** *B. coprophila.*
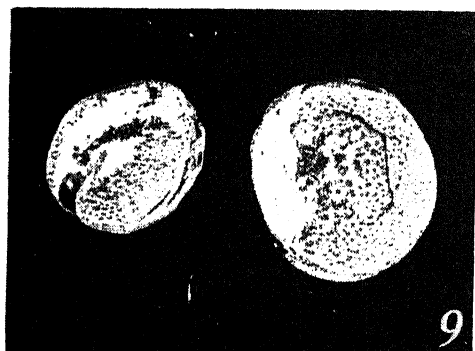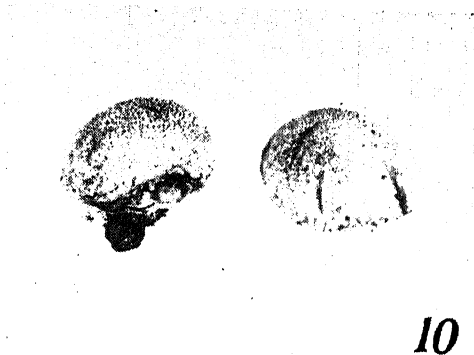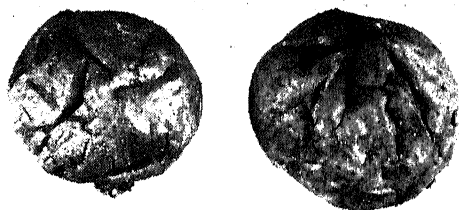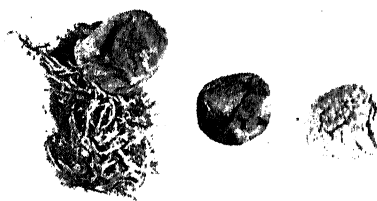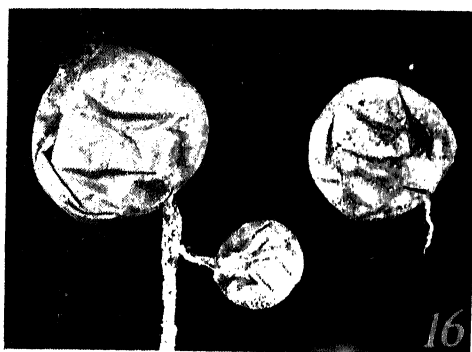
Figures 9–16.   Fructifications.   **9.** *V. endotephrum.*   **10.** *V. curtisii.*   **11.** *M. subincarnata.*
12. *B. fusca.*   **13.** *B. aspera.*   **14.** *B. trachyspora.*   **15.** *B. aestivalis.*   **16.** *B. coprophila.*

Collection examined: Arunachal Pradesh: West Kameng District, Bomdila, Jamiri, on soil near PWD Inspection Bunglow, BMS 23524 (PAN), September 14, 1981.

Remarks: This species was first described from India by Lloyd (1906) as *Bovistella trachyspora* Lloyd, based on Gollan's collection from Respana valley, Mussoorie hills, western Himalaya. Later, Ahmad also collected it from Chamba and Mussoorie hills (western Himalaya) but he transferred it to *Lycoperdon* on the basis of its long, branched, intertwined capillitial threads which are typical of the genus. However, Kreisel (1964) transferred this species to the genus *Bovista* Dill. ex Pers., mainly due to the absence of subgleba and following Kreisel's concept, we have also described this species under the genus *Bovista*.

Arunachal collection is typical of *B. trachyspora* as it agrees very well with Kreisel's description of the species.

2.7 *Bovista aestivalis* (Bon.) Demoulin, Beiheft zur Sydowia, Annals Mycologici Sero. II, **8**: 143, 1979.
Syn. *Lycoperdon aestivale* Bon., Handb. allg. Mykol., 251, 1851 Figures 7 and 15.

Fructifications gregarious, scattered, globose or subglobose, plicating below to the point of attachment, up to 2 cm in diameter, attached to the substratum by short, thread like rhizomorphs. Exoperidium pale brown to shining greyish brown, smooth, membranous, dehiscing by a small apical stoma. Subgleba absent. Gleba olive brown and pulverulent at maturity. Capillitium threads '*Lycoperdon*' type, olive brown, dichotomously branched, branches lighter, tapering into bluntly acuminate apices, aseptate, wall up to 1 μm thick, pitted, pores few, small, up to 4·5 μm wide. Basidiospores globose, 3·5–5 μm in diameter, pale olive brown, verruculose, verrucae minute, distinctly visible in cotton blue, projecting into a thin hyaline envelope, guttulate, usually with a short stump of pedical.

Collections examined: Arunachal Pradesh: West Kameng District, Bomdila, 11 km from Bomdila towards Munna, on humicolous soil, predominantly angiospermous forest, BMS 23458 (PAN), August 28, 1981; 10 km from Rupa towards Shergaon, on humicolous soil, predominantly pine forest, BMS 23487 (PAN), September 4, 1981.

Bhutan: Thimphu, Nawephu, on humicolous soil, mixed forest, BMS 23245 (PAN, LG), September 17, 1980; Namseling, on soil, predominantly pine forest, BMS 23291 (PAN), September 24, 1980.

Remarks: This species has been recorded here for the first time from India and Himalaya. It is a common species in the forest around Thimphu (Bhutan). Eastern Himalayan collections agree well with the concept of the species given by Demoulin (1979) but differ in having smaller fructifications and few small pores in the capillitium in contrast to numerous pores known in *B. aestivalis*. PAN collections appear to be representing a small form of *B. aestivalis*.

2.8 *Bovista coprophila* (Cke. and Mass.) G H Cunn., New Zealand J. Sci. Techn., **23**: 171, 1942. Figures 8 and 16.

Fructifications scattered, singly, subglobose, up to 1·8 cm in diameter, attached to

the substratum usually by a single, thin or thick, long persistent rhizomorphic strand. Exoperidium comprising a thin coat of whitish, conical, mealy squamules, which are larger and dense in the upper part, becoming smaller and scattered below, fugacious at maturity. Endoperidium yellowish grey to light brown, rough, wrinkled, membranous, dehiscing by an apical torn stoma. Subgleba inconspicuous. Gleba olivaceous brown, pulverulent. Capillitium 'intermediate' type, main stem brownish yellow, dichotomously branched, branches lighter, slender with acuminate apices, aseptate, thick-walled, wall up to 3 μm thick, rarely pitted, up to 12 μm wide. Basidiospores subglobose to ovoid, sometimes globose, 5–6 × 4·5–8·5 μm, pale olive brown, verruculose, verrucae distinctly visible in cotton blue, projecting into a thin, hyaline envelope, guttulate, usually with a short stump of pedicel.

Collections examined: Bhutan: Thimpu, Namseling, on soil, BMS 23343 (PAN), September 14, 1980; D Dzong, on soil mixed forest, BMS 23346 (PAN, LG), September 23, 1980.

Remarks: These Bhutan collections are typical of *Bovista coprophila*, which is first report from the Himalaya. The species is marked by subglobose fructifications attached to the substratum by a single persistent rhizomorph, 'inter-mediate'-type of capillitium, inconspicuous subgleba and subglobose to ovoid basidisopores with a short stump of pedicel.

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Seedborne nature of *Peronospora parasitica* in *Raphanus sativus*

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Abstract. The alkali maceration technique was used to detect the seedborne nature of *Peronospora parasitica* in *Raphanus sativus*. Four cultivars 'Japanese white', 'Arka nishant' 'Pusa desi' and 'Pusa reshmi' were used to confirm the presence of pathogen in the seed. The percentage of embryonal infection in the cultivars were 12·5, 0·5, 0·25 and 0·1 respectively. The percentage of seedling infection is directly correlated to the percentage of embryo infection. The possibility of using this technique in quarantine screening is discussed.

Keywords. Radish; embryo; pericarp; internal inoculum; seedborne.

1. Introduction

Peronospora parasitica (Pers. ex. Fr.) Fr. causes downy mildew in many Cruciferous hosts. In many situations, partial or complete destruction of some leaves, is the total expression of the disease in the field, but in certain crops such as cauliflower and broccoli the infection may extend to the curds both in field (Chorin 1946; Davison *et al* 1962; Jenkins 1964; Shiraishi *et al* 1975) and in store (Lund and Wyatt 1978). The radish downy mildew pathogen has attracted few workers (Baudys 1928; Shiraishi *et al* 1975; Sharma and Sohi 1982). The seedborne nature of the fungus has not been established. Hence the present study was set out to detect the seedborne nature of the pathogen and the percentage of viable inoculum in the seeds.

2. Materials and methods

Four cultivars 'Japanese white', 'Arka nishant', 'Pusa desi' and 'Pusa reshmi' were sown in the field at Downy Mildew nursery at Mysore.

Seeds (400) from each cultivar were sown in separate plots which were observed periodically for the occurrence of downy mildew disease. At the seed setting stage, seeds from infected plants were subjected to maceration technique (Shetty *et al* 1978). Seeds were placed in 250 ml of 10% NaOH for 24, 36, and 48 h respectively, at 22°C along with 0·5 g of Trypan blue stain. After the alkali treatment the seeds were agitated in warm water (60–70°C) for 5 min. Hard seeds were softened by boiling in 5% NaOH for an additional 5–10 min. Seeds were then sieved, excess water drained off and lactophenol added to a beaker containing the treated seeds. The lactophenol completed detachment of the embryo from the seed coat. The beaker with the embryos and the seed coats was placed in water bath and heated with low flame until the embryos were cleared. The embryos and seed coats were examined under stereomicroscope.

To determine the viability of the internally borne mycelium, seedling symptom test was carried out. Seeds (400) from the above samples were sown under control conditions in a glass house which is free from airborne inoculum. Before sowing, the seeds were surface sterilised using 0·1% mercuric chloride solution for 5 min, followed by 5 washings in sterile distilled water. Such seeds were sown in pots containing steam sterilized soil (20 pound pressure for 15 min). After seedling emergence, observation was made daily and disease incidence was recorded. Number of seedlings infected from each cultivar was recorded. The seeds from the first harvest were subjected to alkali maceration technique to determine the rate of transmission of the pathogen in the seeds.

3. Results

The results of the maceration technique showed the presence of mycelium in the
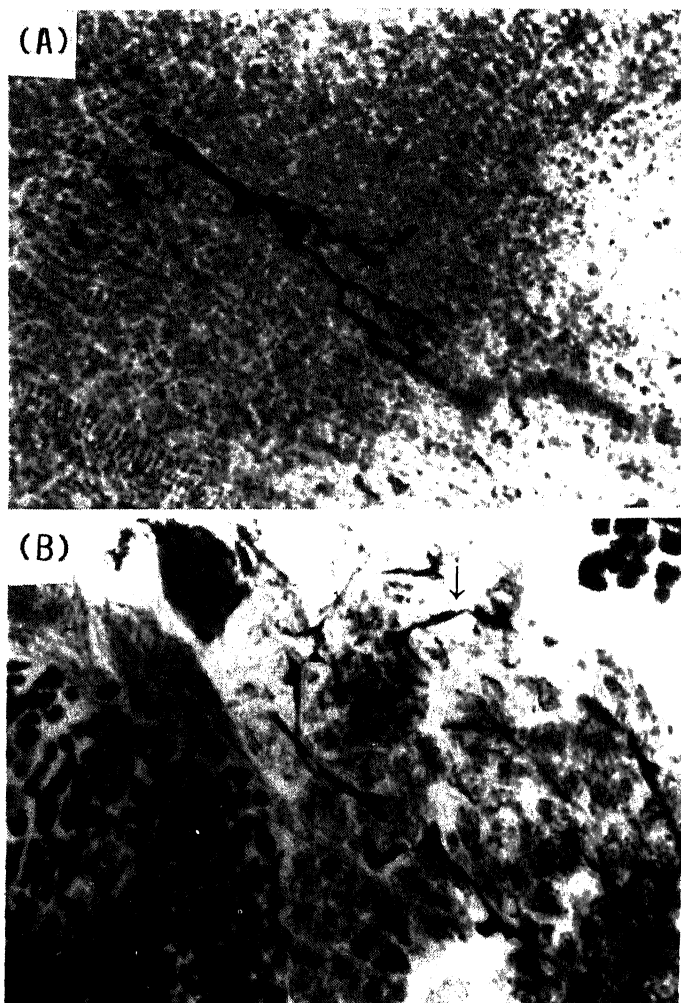
Figure 1. Mycelium of *P. parasitica* in radish seeds. (A) Pericarp (× 200). (B) Embryo (× 200).

pericarp and embryo. The coenocytic branched mycelium was clearly visible in the intercellular space of the pericarp (figure 1A). In the embryonal tissues, the mycelium was comparatively thin (figure 1B). No mycelium was observed in the endosperm of the infected seeds. Percentage of embryonal infection varied from cultivar to cultivar (table 1).

The seedling symptom test showed that the internally borne mycelium resulted in the infection of the seedlings. Percentage of seedlings infection varied from cultivar to cultivar and the viable inoculum transmitted the pathogen to the seeds (table 2).

4. Discussion

Few reports on the biology and host-parasite interaction of *P. parasitica* among Crucifers are known (Chang *et al* 1963; Chou 1970; Greenhalgh and Dickinson 1975; McMeekin 1981) and no information was available regarding its seedborne nature in radish. In the present study, the seedborne nature of the pathogen has been well established in radish. The detection of internal inoculum in the seeds has given light to the abundant and frequent occurrence of the downy mildew disease in radish cultivated areas and its introduction in new areas at Mysore.

The percentage of seeds with viable mycelium is directly correlated with the percentage of embryonal infection. In the seedling symptom test, the transmission rate of the pathogen in the seed is also correlated to the percentage of embryo infection. The presence of *P. parasitica* in radish seeds should be of grave concern to the quarantine authorities. There are several instances where pathogens have been introduced from one country to another through seeds (Neergaard 1977).

Table 1. Percentage seed infection by *P. parasitica* in *R. sativus*.

Cultivar	Place of collection	Seed showing infection (%)		
		Pericarp	Endosperm	Embryo
Japanese white	Mysore Seed Multiplication Farm	12·8	0	12·5
Arka nishant	Indian Council of Agricultural Research Station, Bangalore	0·5	0	0·5
Pusa desi	Bangalore Seed Health Testing Station	0·21	0	0·25
Pusa reshmi	—"—	0	0	0·1

Table 2. Percentage of seedling infection by *P. parasitica* and seed transmission in *R. sativus*.

Cultivar	Seedling infection (%)	Seed infection (%)	
		Pericarp	Embryo
Japanese white	14·0	13·5	12·8
Arka nishant	1·5	0·5	0·4
Pusa desi	1·0	0·1	0·15
Pusa reshmi	1·0	0	0·1

Xanthomonas campestris, causing black rot of Crucifers, resulted in severe epidemic in USA by seeds imported from Europe and *P. farinosa* in beet was introduced to Australia in the form of oospores carried along the imported seeds. The maceration technique is a simple, quick, economical and reliable technique for the detection of internal inoculum in Cruciferous seeds.

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Occurrence of *Sclerocystis* species in semi-arid soils of India

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Abstract. Three species of a vesicular-arbuscular mycorrhizal fungus, *Sclerocystis*, viz., *Sclerocystis pakistanica* Iqbal and Bushra, *Sclerocystis clavispora* Trappe, and *Sclerocystis sinuosa* Gerdemann and Bakshi, have been found to occur consistently in the agricultural fields planted to sorghum and foxtail millet in Anantapur district of Andhra Pradesh. A comparison of the morphological features of these sporocarps was made with those already reported.

Keywords. Foxtail millet; *Sclerocystis* spp.; semi-arid soil; sorghum; VAM fungus.

1. Introduction

Most members of the Endogonaceae have been described and identified based on the morphology of their sporocarp and/or spores (Gerdemann and Trappe 1974; Hall 1984). Despite the interest in vesicular-arbuscular mycorrhizal (VAM) research during the past decade, only a few studies have included tropical soils of India. Moreover, a perusal of the literature on VA mycorrhiza reveals no information on the ecological distribution of *Sclerocystis* spp. (Hetrick 1984). Again, excepting the recent report (Ammani *et al* 1986), there has been no survey in India on the occurrence of *Sclerocystis* spp. This paper describes the occurrence and distribution of *Sclerocystis* spp. in semi-arid soils of Anantapur district in Andhra Pradesh, India.

2. Materials and methods

Sorghum and foxtail millet fields from Anantapur, Atmakur, Jangalapalli, Miduthur, Mustur and Yerraguntapalli in Anantapur district were selected for the present survey. Root samples, collected at regular intervals, were washed gently and stained with trypan blue (Phillips and Hayman 1970). Sporocarps from rhizosphere and non-rhizosphere soil samples were extracted following the wet-sieving and decanting technique of Gerdemann and Nicolson (1963).

3. Results and discussion

VAM fungi were widespread and sporocarps of *Sclerocystis* spp. were noticed in different agricultural fields of Anantapur district. These sporocarps were identified by referring to the slide collection along with the index provided by Hall and Abbott (1981).

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In all, three species of *Sclerocystis* were consistent in their occurrence. Of these, *S. pakistanica* was the most predominant while *S. clavispora* was noticed in a few places of the district and *S. sinuosa* occurred infrequently in cultivated soils. The sporocarpic populations of *Sclerocystis* were widely distributed in the fields of foxtail millet than those of sorghum. The total individual chlamydospores of *Sclerocystis* spp. extracted by wet-sieving method were less in number compared to other species populations of VAM fungi. Thus, about 2–7% of the total spore population of VAM fungi in root zone soils comprised chlamydospores of *Sclerocystis* spp. However, no clear relationship could be established between spore number and root colonization by species of *Sclerocystis*.

3.1   *S. pakistanica*

The sporocarps of the present isolate were almost similar to those reported by Iqbal and Bushra (1980). However, the cylindroclavate chlamydospores showed variation in that the walls were uniformly thin except at the base (table 1), and were not parasitized (figure 1). Endospores were absent in chlamydospores. The peridium enclosed more than 100 chlamydospores and was found to contain a clear palisade-like hyphal arrangement (figure 2) as against the thick-walled interwoven hyphal peridium reported from Pakistan.

3.2   *S. clavispora*

S. clavispora was second to *S. pakistanica* in its abundance. The sporocarps resembled those reported by Trappe (1977) but were slightly larger in diameter compared with the collection of Iqbal and Bushra (1980) from Pakistan. Further, the presence of a well developed peridium (table 1) conforms to the report of Ammani *et al* (1986). The arrangement of chlamydospores inside the peridium was radial around a central plexus consisting of tightly interwoven pale brown thin-walled hyphae (figure 3). Chlamydospores were orangish-brown, cylindric, subcylindric to clavate (figure 4), slightly tapering towards the base and open through a narrow pore into the thick-walled subtending hyphae.

Table 1.   Particulars of the sporocarps of *Sclerocystis* spp. isolated from millet fields.

Parameter	*S. pakistanica*	*S. clavispora*	*S. sinuosa*
Diameter of sporocarp (μm)	561·3–596·4	613·9–684·1	230·0–250·0
Width of the peridium (μm)	40·3	3·0–4·5	4·3
Diameter of central plexus (μm)	221·0–255·0	213·0–246·5	160·0–182·8
No. of chlamydospores	100	80	15–20
Size of chlamydospores (μm)	114·7–127·5 × 46·0–76·0	148·7–161·5 × 21·3–40·3	45·0–95·0 × 38·0–80·0
Thickness of internal walls (μm)	2·0	3·0–4·5	4·0–4·3
Thickness of chlamydospore walls at the base (μm)	4·3	7·0–8·5	8·0–10·5
Length of subtending hyphae (μm)	34·0	3·0	12·8
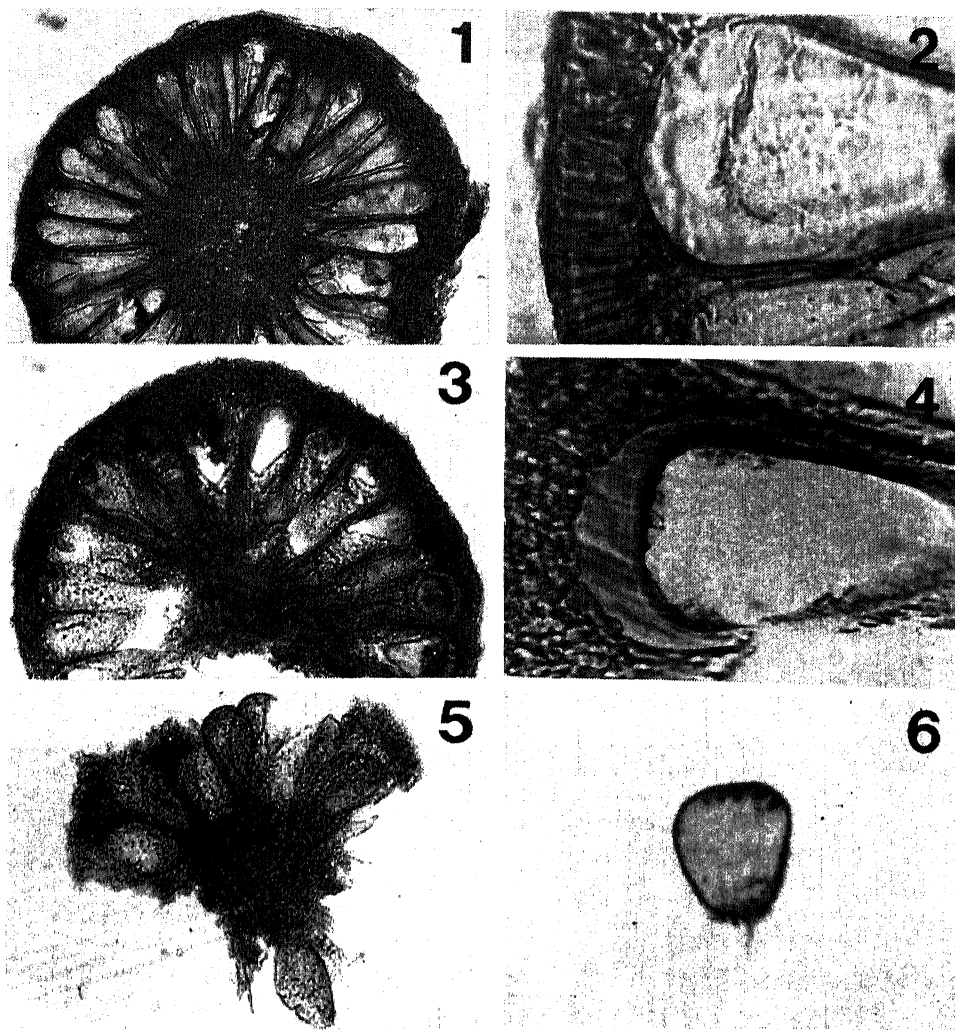
Figures 1–6. **1.** Section of *S. pakistanica* sporocarp (× 320). **2.** Single chlamydospore of *S. pakistanica* showing palisade-like peridial structure (× 1200). **3.** Section of *S. clavispora* (× 320). **4.** Single chlamydospore of *S. clavispora* showing inter-woven hyphae of the peridium (× 1200). **5.** Section of *S. sinuosa* sporocarp (× 640). **6.** Single parasitized chlamydospore of *S. sinuosa* (× 1200).

3.3   *S. sinuosa*

These sporocarps were also found associated externally with the root in the rhizosphere soil. The sporocarps were brown, globose to subglobose with much smaller size than those of *S. pakistanica* and *S. clavispora*. The peridium was composed of thick-walled sinuous hyphae (figure 5), tightly enclosing squarish chlamydospores. Occasionally, chlamydospores were found parasitized (figure 6) and laminations occurred rarely in the spore wall.

The present investigation clearly revealed the occurrence of *Sclerocystis* spp. in

semi-arid soils of Andhra Pradesh. It is worthwhile to determine the impact of these endophytes on economically important millet crops.

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Distribution of vegetation types in northwest Himalaya with brief remarks on phytogeography and floral resource conservation

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Abstract. The vegetational wealth of northwest Himalaya is discussed in this paper. Unlike the vegetation of eastern Himalaya, the forests are not diverse and rich. The forests here are mainly classified under (i) tropical forests, (ii) subtropical forests, (iii) temperate forests, (iv) subalpine forests and (v) alpine vegetation, primarily based on the altitude. The plant resources of the region are briefly outlined with reference to (i) wild edible plants, (ii) medicinal and aromatic plants, (iii) ornamental plants, (iv) orchids, (v) fodder resources, (vi) bamboos and (vii) other biologically interesting species. The brief phytogeographical affinities of the northwest Himalayan flora, the major threats to the flora and some conservation programmes are also discussed.

Keywords. Northwest Himalaya; plant resources; affinities; threats and conservation.

1. Introduction

Although the Himalaya form a continuous chain of mountain system the distribution pattern of vegetation varies significantly from west to east. The eastern Himalaya are more green and diverse compared to the dry arid regions in the western Himalaya. The northwest Himalaya for this study comprises the areas of Jammu and Kashmir, Himachal Pradesh and western Uttar Pradesh.

2. Vegetation

The vegetation type met with in any particular area depends on the climate, the soil, topographical situation and geographical location. The topography of northwest Himalayan region is irregular and disturbed by valleys and plateau of various extent and as such the stratification is not clear. There is also a great diversity in the floristic pattern due to great altitudinal variation, coupled with rainfall factor which becomes lesser and lesser as one travels from east to west. However, on the basis of altitude and climate the vegetation types of this region may be divided as follows:

1. Tropical forests:
   - (i)   Scrub forests
   - (ii) Deciduous forests
   - (iii) Tree savannah forests
   - (iv) Swamp forests

2. Subtropical forests:
   - (i)   Broad leaved forests
   - (ii) Pine forests
   - (iii) Subtropical evergreen sclerophyllous forests

3. Temperate forests:
   (i)   Broad leaved forests
   (ii) Coniferous forests

4. Sub alpine forests

5. Alpine vegetation.

The details of the floristic composition and distribution of these forests are avoided as the same are discussed in detail by Schweinfurth (1957), Gupta (1964), Champion and Seth (1968), Rau (1974), Dhar and Kachroo, (1983) and Singh and Singh (1987).

3. Affinities

The study on the phytogeographical affinities of the flora of the northwest Himalaya with the surrounding regions is indeed very fascinating. The close affinity between the flora of the northwest Himalaya with those of Europe, the near east and middle east is well established (Legris 1963; Gupta 1962, 1964, 1982; Meher-Homji 1973; Rau, 1974, 1975, 1981; Dhar 1978; Sahni 1982).

The European and central Asian elements are frequent in areas west of the river Sutlej, while the Chinese elements extend from Yunnan in the east right through the east Himalayan ranges.

From the dry mountains of western and middle Asia many elements have spread to the western ranges of the Himalaya. This influx is greatly due to the arid and dry conditions prevailing here particularly in the interior ranges of Ladakh, Lahul and Spiti valley. Several such species of middle Asia like *Rosularia alpestris, Salix karelinii, Sorbaria tomentosa, Lathyrus humilis, Acantholimon lycopodioides, Myricaria squamosa, Oxytropis microphylla, Halogeton glomeratus, Biebersteinia odora* etc. are found in the northwest Himalayan region.

Cedrus deodara common on the west Himalayan slopes is also distributed as far away as in Afghanistan. The eastern limit of the distribution of the species is the western part of Nepal. Based on this as well as on the distribution pattern of several species it has been concluded that the zone of transition between the phytogeographical regions of eastern and western Himalaya is approximately the area between 80°E to 84°E longitude (Stearn 1960; Banerji 1963).

As far as number of gymnosperm species is concerned, although the eastern Himalaya is richer there exist vaster, coniferous forests in the western Himalaya. *Pinus gerardiana, Juniperus polycarpos, Picea smithiana* are among the gymnosperms distributed in the northwest Himalaya but absent in the eastern Himalaya. Similarly *Ephedra*, an important genus of medicinal value, is well represented in the northwestern Himalaya with 6 species while only one species occurs in the eastern Himalaya.

Several species like *Larix griffithiana, Picea spinulosa, Cephalotaxus griffithii, Gnetum montanum, Cycas pectinata* etc. found in east Himalaya are absent beyond east Nepal.

Several temperate species from Europe and other temperate regions have also found their way to this region. Some of these species are *Melilotus officinalis, Medicago falcata, Aconogonum alpinum, Trifolium repens, Lotus corniculatus,*

Onopordum acanthium, Chenopodium foliosum, Centaurea iberica, Geranium pratense, Mentha longifolia, Carthamus lanatus, Artemisia absinthium, Briza media, Dactylis glomerata, Poa trivialis, Draba nemorosa, Erophila verna, Barbaraea vulgaris, Cardamine impatiens etc.

There are also other introductions like *Datura sauveolens, D. stramonium* from tropical America, *Nicandra physaloides* from Peru, *Ipomoea purpurea* from central America, *Ipomoea carnea* from south America, *Martynia annua* from America etc. which have now become naturalized.

Viola biflora a common species in the northwestern Himalaya is also known from Europe, Siberia, central Asia, north Korea, Japan, north America as well as in the central and eastern Himalayan regions. Similarly *Capparis spinosa* is known from Afghanistan to Nepal, West Asia, Europe. *Poa alpina* is another species which is widely distributed in Pakistan, India, Europe, Mediterranean region, middle east to central Asia and north America.

However, some species like *Cotoneaster frigidus, Rubus calycinus, Rubus acuminatus, Androsace delavayi, Osmanthus suavis, Boschniakia himalaica* etc. originating in southwest China reach only up to Kumaon in Uttar Pradesh. Similarly there are species like *Cypripedium elegans, Cypripedium himalaicum, Roscoea purpurea, Primula tibetica, Primula primulina* etc. which extend from southwest Tibet to Uttar Pradesh.

Circaeaster agrestis another plant of north-western China extends across Tibet to the Himalaya as far west as Garhwal. Similarly there are several species distributed not only in the northwestern Himalaya but all along the Himalayan range up to southeast Asia, Burma, etc. (table 1).

The extraneous elements of the flora from southwest China, central Asia, west Asia, Europe have mixed-up with the local species now forming a permanent

Table 1. Some species of Chinese origin widely distributed in the Himalaya.

Name	Family	Distribution
Valeriana jatamansii Jones	Valerianaceae	Afghanistan to southwest China, Burma
Valeriana hardwickii Wall.	Valerianaceae	Pakistan to southwest China, Burma, southeast Asia
Cardiocrinum giganteum (Wall.) Makino	Liliaceae	Kashmir to southwest China, Burma
Dactylorhiza hatagirea (D Don) Soo	Orchidaceae	Pakistan to southeast Tibet, Europe, north Africa, central west and southeast Asia
Primula denticulata Smith	Primulaceae	Afghanistan to southeast Tibet, Burma
Taxus baccata L. ssp. *Wallichiana* (Zucc.) Pilger	Taxaceae	Afghanistan to southwest China, Burma, southeast Asia
Symplocos paniculata (Thunb.) Miq.	Symplocaceae	Pakistan to southwest China, Burma, Japan, southeast Asia
Jasminum dispermum Wall.	Oleaceae	Kashmir to southwest China, southeast Asia
Buddleja asiatica Lour.	Loganiaceae	Pakistan to Bhutan, central and south China, Burma, southeast Asia
Acer oblongum Wall. ex DC.	Aceraceae	Pakistan to southwest China, Burma, southeast Asia
Hedera nepalensis K Koch	Araliaceae	Afghanistan to southwest China, Burma
Svida oblonga Sojak	Cornaceae	Kashmir to southwest China, Burma, southeast Asia
Leycesteria formosa Wall.	Caprifoliaceae	Pakistan to southwest China, Burma
Lonicera webbiana Wall. ex DC.	Caprifoliaceae	Afghanistan to southwest China

denizen of the northwestern Himalayan flora. At the same time, some of these migratory elements have remained unchanged. Example of such species are *Melilotus alba, Melica nutans, Potentilla fruticosa, Aconogonum alpinum, Flemingia strobilifera, Nasturtium officinale*, etc.

Some species are also treated as related sub-species or close varients of the species found in Eurasian regions. Presumably these taxa migrated to this region during the Pleistocene glaciations and subsequently adopted to the new environs resulting in their present status.

Although the Himalaya form one continuous chain of mountains running from Naga Parbat on the Indus to Namcha Barwa on the bend of the Tsang-Po in south east Tibet (*ca* 2250 km), the floristic patterns of west Himalaya are so distinct that it is impossible to treat them as one unit, atleast botanically. The eastern Himalayan region is richer and more diverse in plant wealth because of the greater amount of precipitation which the area receives compared to western Himalaya.

The distribution and strength of some taxa in western and eastern Himalaya are shown in table 2.

Although the northwest himalayan flora is an admixture of floras from Mediterranean region, central Asia, Europe, southwest China, etc., a careful analysis reveals that the northwest Himalayan region is also rich in endemic species (table 3).

4. Plant resources

The rich plant wealth of northwest Himalaya have sustained numerous tribal populations for centuries. These tribals (Ladakhis, Mirbahris and Gujars of Jammu and Kashmir, Gaddis, Lahuliaas of Himachal Pradesh, Jaunsari of Jaunsar Bawar, Bhottyas of the border districts of Uttar Pradesh) have their own way of association with the flora and fauna of the region. It is however not possible to highlight all groups of economic plants in this paper but only some important groups which can form a stable source of revenue to the states if properly utilised.

4.1 *Wild edible plants*

The tribals and other villagers in the Himalaya, consume certain plants or plant parts as vegetables or otherwise. However they have not attempted to undertake large scale cultivation of these edible plants and they rely on the forests around them for their supply of these plants.

These non traditional food plants are, however, not known in other parts of the

Table 2.

Taxa	Western Himalaya (No. of species)	Eastern Himalaya (No. of species)	India (No. of species)
Orchids	*ca* 250	*ca* 640	*ca* 1100
Rhododendrons	*ca* 5	*ca* 82	*ca* 82
Bamboos	*ca* 19	*ca* 58	*ca* 100
Hedychiums	*ca* 7	*ca* 34	*ca* 35

Table 3. Some endemic species in northwest Himalaya.

Species	Altitude (m)	Distribution
Androsace primuloides Duby	3000–4000	Jammu and Kashmir
Hedysarum cachemirianum Benth. ex Baker	2500–4000	Jammu and Kashmir
H. microcalyx Baker	2500–400	Jammu and Kashmir to Uttar Pradesh
Saussurea atkinsonii Clarke	3000–4500	Jammu and Kashmir to Uttar Pradesh
Saussurea clarkei Hook.f.	*ca* 4400	Jammu and Kashmir
Poa falconeri Hook.f.	*ca* 4000	Jammu and Kashmir
Poa koelzii Bor	*ca* 5000	Jammu and Kashmir
Puccinellia stapfiana R R Stewart	*ca* 5000	Jammu and Kashmir
Puccinellia thomsonii (Stapf) R R Stewart	*ca* 5000	Jammu and Kashmir
Catabrosella himalaica (Hook.f.) Tzvelev	*ca* 4500	Jammu and Kashmir
Arabis tenuirostris D E Schulz	*ca* 3000	Jammu and Kashmir
Hyalopoa nutans (Stapf) Alexeev	*ca* 3500–4500	Jammu and Kashmir
Delphinium roylei Munz	*ca* 1600–2500	Jammu and Kashmir
Carex munroi Boot ex Clarke	*ca* 3800	Himachal Pradesh
Microschoenus duthie Clarke	*ca* 5300	Uttar Pradesh
Dicranostigma lactucoides Hook.f. and Thoms.	*ca* 2700–4000	Jammu and Kashmir Himachal Pradesh
Erophila tenerrima (E Schulz) Jafri	*ca* 4200	Jammu and Kashmir
Christolea scaposa Jafri	*ca* 4950	Jammu and Kashmir

country. It is also true that the known traditional food plants may not be sufficient to feed the growing population in the years to come. Therefore study on the food value of wild species is highly essential. A few important wild food plants locally available are listed. It is essential to commercialise a few of the species at least on a regional basis (table 4).

4.2 *Medicinal and aromatic plants*

Medicinal virtues of western Himalayan plants are well known from the early times of the great epics of Ramayana and Mahabhartha. The high hills are the storehouse of numerous bearing herbs which are exploited not only for the pharmaceutical industries in India but outside as well. Due to the unscrupulous traders several of these medicinal species have already become rare in their natural habitats. *Colchicum luteum* of Jammu and Kashmir and Himachal Pradesh, the Brahma Kamal (*Saussurea obvallata*) in the Garhwal Himalaya, the Indian Belladona (*Atropa acuminata*) of Jammu and Kashmir and Himachal Pradesh are only some cases which can be mentioned. Some of the medicinal plants are highly priced for example Angelica (*Angelica glauca*) costs Rs 2/- per 10 g in the local market, similarly 40 kg of Kuth (*Saussurea costus*) costs Rs 1,200. Naturally, there is temptation both among the locals as well as outsiders for collection of these plants. Although some species are brought under cultivation, several other species are being exploited from their natural habitats.

Same is the case with regard to certain aromatic plants which are extensively used in perfume industry, etc. Large scale cultivation of these plants in this region can be highly regarding. Some of the important medicinal and aromatic species which can be profitably exploited under large scale cultivation are listed in table 5.

Table 4.   Some wild edible plants.

Name	Family	Vernacular name	Parts used	Distribution
Nymphoides peltata (S Gmelin) Kuntz	Menyanthaceae	Water Chest nut 'Khur'	Nuts	Common in Kashmir valley Temperate Eurasia
Euryale ferox Salisb.	Nymphaeaceae	'Jewar'	Seeds	Kashmir to Assam, China
Nymphaea stellata Willd.	Nymphaeaceae	Bumbosh	Tuber	Throughout India, Africa
Nelumbo nucifera Gaertn.	Nymphaeaceae	Indian lotus	Nuts and petiole	Throughout India, common in Dal Lake, Kashmir, north China
Allium carolinianum DC	Amaryllidaceae	Wild onion	Leaves	Afghanistan to central Nepal, 3300–4800 m
Allium rubellum M Bieb	Amaryllidaceae	Wild onion	Bulbs	Kashmir
Podophyllum hexandrum Royle	Berberidaceae	May-apple	Ripe fruits	Afghanistan to southwest China
Pinus gerardiana Wall. ex. Lamb.	Pinaceae	Chilgoza	Seeds	Afghanistan to Uttar Pradesh
Dactylorhiza hatagirea (D Don) Soo	Orchidaceae	Sallam Panza	Tubers	Pakistan to southeast Tibet
Cicer microphyllum Benth.	Papilionaceae		Young shoots	Afghanistan to west Nepal, 3300–4500 m
Rheum webbianum Royle	Polygonaceae	Rhubarb	Leaves	Pakistan to west Nepal, 2500–4200 m
Rhodiola imbricata Edgew.	Crassulaceae		Stem and leaves	Pakistan to central Nepal *ca* 4500 m
Rubus ellipticus Smith	Rosaceae	Wild raspberry	Fruits	Pakistan to southwest China, south India, Sri Lanka, southeast Asia, 600–2000 m
Fragaria nubicola Lindl. ex Lacaita	Rosaceae	Wild strawberry	Fruits	Pakistan to southwest China, Burma, 1800–3800 m
Nasturtium officinale B Br.	Brassicaceae	Water-cress	Leaves	Afghanistan to Arunachal Pradesh, Temperate, Asia, Europe, north Africa, 1500–3500 m
Dendrocalamus strictus (Roxb.) Nees	Poaceae	Bamboo	Young shoots	Pakistan to Burma up to 1000 m
Eremurus himalaicus Baker	Liliaceae	Desert candle	Leaves	Afghanistan to Himachal Pradesh, central Asia, 2000–3000 m
Bombax ceiba L.	Bombacaceae	Silk cotton	Flower buds	Jammu and Kashmir to Bhutan, south China, southeast Asia, *ca* 1000 m

Table 4. (*Contd.*)

Bauhinia variegata L.	Caesalpiniaceae	Kachnar	Flower buds	Arunachal Pradesh, Pakistan to Burma, China, *ca* 1500 m
Opuntia monacantha (Willd.) Haw.	Cactaceae	Nagphal	Ripe fruits	Native of south America (planted as hedge plant and ripe fruits are sold in the market)
Urtica dioica L.	Urticaceae	Stinging Nettle Bichhu	Young leaves and top of branches	Pakistan to south-west China, up to 2500 m
Myrica esculenta Buch.-Ham. ex D Don	Myricaceae	Kaphal	Fruits	Jammu and Kashmir to Bhutan, Burma, China, southeast Asia, 1000–2000 m
Elaeagnus parviflora Wall. ex Royle	Elaeagnaceae	Girvai Goe-win Gehain	Fruits	Afghanistan to south west China, 1000–2500 m
Hippophae rhamnoides L. sub sp. *turkestanica* Rousi	Elaeagnaceae	Tarwa Tasru Sirna	Fruits	Pakistan to Himachal Pradesh, central Asia, 2000–3500 m

Table 5. Medicinal and aromatic plants.

Species	Vernacular name	Ecology	Distribution and altitude
Aconitum heterophyllum Wall. ex Royle	Atis	Open grass-lands	Pakistan to central Nepal, 2500–4000 m
Arnebia benthamii (Wall. ex G Don) Johnston	Balchari	Open hill slopes amidst	Pakistan to west Nepal, 3000–4000 m
Atropa acuminata Royle	Indian Bel-ladona	In the forests (cultivated in Jammu and Kashmir)	Pakistan to Himachal Pradesh
Colchicum luteum Baker	Hirantutiya	Open hill slopes	Pakistan to Himachal Pradesh, 1000–2500 m
Dioscorea deltoidea Wall.ex Kunth	Kins	At the edge of the forests in open places	Jammu and Kashmir to Bhutan, Afghanistan, 2000–2500 m
Gentiana kurrooa Royle	Karu	Open grass lands	Pakistan to Uttar Pradesh, 1800–2500 m
Nardostachys grandiflora DC.	Jatamanshi	In rock crevices and in open places	Uttar Pradesh to south-west China, 3500–4500 m
Picrorhiza kurrooa Royle ex Benth.	Katki	In rock crevices and in open places	Pakistan to Uttar Pradesh, 3000–4000 m
Podophyllum hexandrum Royle	Ban Kakri	Amidst boulders	Afghanistan to southwest China, 2500–4000 m

Table 5. (*Contd.*)

Species	Vernacular name	Ecology	Distribution and altitude
Hedychium spicatum Smith	Spiked ginger lilly	Epiphytic or in the forests	Himachal Pradesh to Arunachal Pradesh 1500–2500 m
Fritillaria roylei Hook.		Amidst grasses in alpine meadows	Pakistan to Uttar Pradesh, 2500–4000 m
Corydalis govaniana Wall.	Bhutan-Keshi	Amidst boulders in the sub-alpine and alpine region	Pakistan to east Nepal, 3500–4000 m
Ferula jaeschkeana Vatke		On open hill slopes	Pakistan to Himachal Pradesh, 2500–3500 m
Prangos pabularia Lindl.	Avipriya	On open hill slopes amidst boulders	Afghanistan to Kashmir, 2000–3000 m
Dactylorhiza hatagirea (D Don) Soo	Salam Panja	In alipine and sub-alpine meadows	Pakistan to southeast Tibet, 3000–4000 m
Rubia manjith Roxb. ex Fleming	Majith	In open places amidst shrubs	Pakistan to southeast Tibet, 1500–2500 m
Valeriana jatamansii Jones	Indian vallerin	Amidst boulders and in the forests	Afghanistan to southwest China, Burma, 1500–3500 m
Arctium lappa L.		In waste places and near the cultivated land	Afghanistan to Nepal, west Tibet
Aconitum deinorrhizum Holms ex Stapf	Safed Bikh	Amidst boulders, on open hill slopes	Jammu and Kashmir to Bhuntan, 2500–3500 m
Artemisia brevifolia Wall.	Kirmala worm seed	In open places, amidst stones	Nepal to Tibet, 2000–4000 m
Berberis aristata DC	Rasaut	In open places	Himachal Pradesh to Nepal
Hyoscyamus niger L.	Henbane Khurasani ajvayan	Along the road side in open places	Pakistan to Uttar Pradesh, southwest China, north Africa, north America, Temperate Eurasia
Jurinea dolomiaea Boiss.	Doop	Open hill slopes	Pakistan to east Nepal, 3000–4000 m

4.3 *Ornamental plants*

This is one group which has not received due attention in India. Although several wild beautiful plants in the Himalaya have been recognised, no efforts have been made to systematically identify, collect, multiply and popularise them in our manmade settings. A few can also be used for improving species already found in our gardens. Only some of the very important ones in this direction are listed in table 6. While these species are suitable for high altitude gardens, their introduction at comparatively lower elevation is possible only after acclimatization trials.

Table 6. Ornamental plants.

Name	Altitude (m)	Flowering time and flower colour	Distribution
Arisema propinauum Schott (Araceae)	2400–3600	May–June, dark purple or green with purple stripes	Jammu and Kashmir, Himachal Pradesh, Uttar Pradesh, Nepal, southeast Tibet
Aster falconeri (Asteraceae)	3000–4200	July–Aug, purple	Jammu and Kashmir, Himachal Pradesh, Pakistan, Nepal
Begonia picta (Begoniaceae)	600–2800	July–August, pinish-white	Jammu and Kashmir, Uttar Pradesh, Bhutan
Berberis lycium (Berberidaceae)	1500–3000	April–June, yellow	Jammu and Kashmir, Uttar Pradesh, Pakistan, Nepal
Capparis spinosa (Capparidaceae)	2000–3000	May–Sept., white	Jammu and Kashmir, Himachal Pradesh, Nepal, west Asia, Europe
Cardiocrinum giganteum (Liliaceae)	2000–3000	June–July, white	Throughout Himalayas, southwest China, Burma
Carissa opaca (Apocynaceae)	600–1200	March–April, white	Jammu and Kashmir, Himachal Pradesh, Uttar Pradesh
Cyananthus lobatus (Campanulaceae)	3500–4500	July–Sept., bright-blue	Himachal Pradesh, Uttar Pradesh, Nepal southwest China
Eremurus himalaicus (Liliaceae)	2000–3000	May–June, white	Jammu and Kashmir, Himachal Pradesh, Afghanistan, Pakistan, Central Asia
Gentiana stipitata (Gentianaceae)	3500–4000	Aug.–Sept., pale-mauve	Uttar Pradesh, Nepal
Hypericum hookerianum (Hypericaceae)	1500–2500	July–Sept., yellow	Uttar Pradesh, Sikkim, Nepal, Bhutan
Impatiens sulcata (Balsaminaceae)	1500–3500	July–August, purple	Jammu and Kashmir, Uttar Pradesh, Himachal Pradesh, Sikkim, Nepal, Bhutan
Inula grandiflora (Asteraceae)	2000–3500	Aug.–Sept. yellow	Jammu and Kashmir, Himachal Pradesh, Uttar Pradesh

4.4 *Orchids*

Much has been said about the orchid wealth in the Himalaya and their depletion from the natural habitats. In India there exist *ca* 1100 species of which 250 species are reported from the hill districts of Uttar Pradesh, Himachal Pradesh and Jammu and Kashmir. They are generally found in the humid tropical to almost alpine regions. It may be mentioned here that out of 250 species of orchids found in the northwestern Himalaya almost 50% of the species are threatened due to disturbance of natural habitats. Some important ornamental orchid species which are also rare in the region are listed in table 7.

Table 7. Ornamental orchids.

Name	Habitat	Distribution
Anoectochilus roxburghii (Wall.) Lindl.	Terrestrial	Uttar Pradesh, northeastern India, Sikkim, Bangladesh, Nepal, Bhutan, Burma, China, Malay, Thailand
Calanthe alpinae Hook.f.	Terrestrial	Uttar Pradesh, Sikkim, Arunachal Pradesh, Nepal, Bhutan
Calanthe pachystalix Rchb.f. ex Hook.f.	Terrestrial	Uttar Pradesh, Nepal, Indo-China
Calanthe plantaginea Lindl.	Terrestrial	Himachal Pradesh, Uttar Pradesh, Arunachal Pradesh, Sikkim, Nagaland, Nepal, Bhutan
Calanthe puberula Lindl.	Terrestrial	Himachal Pradesh, Uttar Pradesh, Arunachal Pradesh, Sikkim, Meghalaya, Nagaland, Nepal, Bhutan, Burma
Cymbidium iridioides D Don	Epiphytic	Uttar Pradesh, Sikkim, Meghalaya, Nepal, Bhutan
Cymbidium lowianum Rchb.f.	Epiphytic	Uttar Pradesh, Arunachal Pradesh, Nagaland, Burma, Thailand
Cypripedium cordigerum D Don	Terrestrial	Uttar Pradesh, Sikkim, Nepal, Bhutan
Cypripedium elegans Rchb.f.	Terrestrial	Uttar Pradesh, Sikkim, Nepal, Bhutan
Cypripedium himalaicum Rolfe	Terrestrial	Uttar Pradesh, Sikkim, Nepal, Bhutan, southeast Tibet, China
Dendrobium heterocarpum Wall. ex. Lindl.	Epiphytic	Uttar Pradesh, northeast India, Sikkim, Nepal, Bhutan, Burma, Java, Srilanka

4.5 *Fodder resources*

Almost all tribal population in the northwest Himalaya rear animals like sheep, goats or even yaks as in Ladakh. These animals consume besides grasses and sedges several other herbs as well as foliage of trees and shrubs. There are several high altitude pasture lands which are being grazed by these flocks for innumerable generations. However, now with the declaration of some areas as National Parks, sanctuaries they are deprived of some of the best pastures known to them. The needs of the tribals, however, cannot be ignored and alternative measures for providing them with adequate fodder must be considered. Some species of high fodder value must be raised near villages as part of social forestry and agro forestry programmes.

The following are some of the important fodder crops which can be raised at different altitudinal zones of the north-west Himalaya.

Acacia catechu, Albizia lebbeck, Bauhinia variegata, Dendrocalamus strictus, Ficus religiosa, Grewia oppositifolia, Morus alba, Ougenia oojeinensis, Quercus leucotrichophora, Quercus floribunda, Ziziphus nummularia, Moringa oleifera, etc. and grasses like *Themeda anathera, Chrysopogon fulvus, Bothriocloa pertusa, Poa pratensis, Dactylis glomerata* etc.

4.6 *Ferns and fern-allies*

The northwestern Himalaya is poor in number of species of ferns and fern-allies in

comparison to eastern Himalaya. Out of *ca* 1000 species of ferns occurring in India only 264 species (Dhir 1979) are reported from this region. Except for the recent report of *Cyathea spinulosa* from Garhwal the tree ferns are not available in this region.

Some of the interesting and rare species of this region are *Botrychium lanuginosum, B. ternatum, B. lunaria, B. virginianum, Osmunda claytoniana, O. regalis, Polystichum atkinsonii, P. duthei, Athyrium duthei, Thelypteris gracilescens, Cheilanthes dalhousiae, C. dubia, Dryopteris gamblei, Woodsia alpina, W. andersonii, W. cycloloba, Selaginella adunca* etc.

4.7 *Bamboos*

The importance of Bamboos is well known. Except the Kashmir valley they are found in almost all states in the tropical to temperate zones up to 3700 m. The western Himalaya is not rich in bamboos. According to Bahadur and Jain (1983) out of *ca* 100 species known from India only 14 species are recorded from the western Himalaya. These species belong to the genera *Bambusa* (4), *Chimonobambusa* (2), *Dendrocalamus* (4), *Phyllostachys* (2) and *Thamnocalamus* (2), *Chimonobambusa jaunsarensis* and *Dendrocalamus hookeri* are coming rare due to over exploitation by the local people.

4.8 *Other interesting plants*

The northwest Himalaya have a number of interesting plants of great scientific curiosity. *Arceuthobium minutissimum*—a tiny loranthaceous parasite on *Pinus*, *A. oxycedri*—parasite on *Juniperus polycarpos, Lathraea squamaria, Boschniakia himalaica, Balanophora involucrata, Aeginetia indica, Orobanche cernua, O. alba* etc. are some of the curious parasitic plants which are rare in the region.

Insectivorous plant species like *Pinguicula alpina, Drosera peltata, Utricularia* spp. etc. are also frequent.

This region also accounts for some primitive plants like *Circaeaster agrestis*—tiny herb with open dichotomous *venation, Parrotiopsis jacquemontiana*—prosenchyma of the wood marked with discs as in coniferae, *Myrica esculenta, Holboellia latifolia* var. *angustifolia, Michelia kisopa* etc.

5. Major threats to flora

The accelerating decline of India's natural wealth during the last few decades is a matter of grave national concern. The reasons for the precarious condition of the natural wealth are too many. Foremost among them is the ever increasing population pressure resulting in the acute need for more and more land both for settlement as well as agricultural purposes. This thirst for land is leading to the reckless destruction of our forests. In the last 30 years about 43 million hectares of forested land have been cleared for developing farmlands in our country. The area under forest cover in India is now estimated to be about 10% only of the total area of India. This is against 33% in the National Forest Policy of India. The northwest Himalaya too like the rest of the country, is no better in this respect.

Of the several factors operating in this region towards depletion of the natural resources the following are some important.

1. Deforestation for (i) extension/development of new townships, (ii) extension of agricultural lands, (iii) for timber and fuel and (iv) for raising monoculture etc.
2. Selective removal of certain medicinal plants such as *Colchicum luteum, Nardostachys grandiflora, Dioscorea deltoidea, Aconitum* spp. etc. in bulk quantities for meeting the needs of the pharmaceutical houses.
3. Falling of certain species for making packing cases for apples, plums etc. which are transported in large quantities.
4. Over collection of orchids and habitat disturbances.
5. Over grazing/lopping of trees for fodder.
6. Construction of artificial reservoirs which lead to submergence of forest areas.
7. Impact of constant tourist and pilgrim activity in such places as Dal lake, Badrinath, Valley of Flowers etc.
8. Road building on hill upsetting the delicate high altitude ecosystem etc.
9. Lime quarrying and setting up of stone crushers in remote forest areas.
10. Establishment of large cement factories which cause great pollution in the area.

6. Some conservation programmes

In the last 2–3 decades there has been a greater realisation of the need to conserve the natural heritage, rather throughout the globe. Several international programmes like the Man and the Biosphere Programme, convention on the International Trade in Endangered Species of Flora and Fauna are all aimed to achieve the conservation programmes. In northwestern Himalayan region the following are some of important conservation measures.
(i) Several protected areas in the form of National Park and Wildlife Sanctuaries have been established in several ecoclimatic zones (table 8). Further the Nanda Devi National Park and Valley of Flowers National Park have also been proposed to be converted to a larger biosphere reserve under the MAB programme. All these pockets form repositories of northwest Himalayan flora and fauna in their pristine and verdant form. However, encroachments by men and cattle are not uncommon and protection of the demarcated areas cannot but be inadequate due to the lack of enough personal and equipment as well as commitments. Greater priority needs to be given to this aspect.
(ii) India is a signatory to Convention on International Trade on Endangered Species (CITES) of plants and animals which had its first conference in Washington in 1973. Under the CITES act the export of some endangered species is strictly banned. Several medicinal plants and orchids are saved from total extinction by the strict application of this convention. Of the several species listed from India the following species are from northwest Himalaya. *Aconitum* spp., *Colchicum luteum, Cyprepedium* spp., *Dioscorea deltoides, Nardostachys grandiflora, Suassurea obvallata, Dianthus cachemericus.*
(iii) Red Data Book deals with plants which are highly endangered and are on way to extinction as is evident by their markedly thin population structure with a low rate of multiplication. All countries have their own Red Data Books and Botanical Survey of India has also brought out the Red Data Book of India. Many species of

Table 8. National parks and sanctuaries in several ecoclimatic zones of northwest Himalaya.

State	Name	District	Area in hectare
Jammu and Kashmir	Dachigam National Park	Srinagar	14,000.00
	Hemis High Altitude National Park	Leh	60,000.00
	Kishtwar National Park	Kishtwar	31,000.00
	Jasrota Wildlife Sanctuary	Kathua	912,800.00
	Lungnag Wildlife Sanctuary	Kargil	40,000.00
	Nandni Wildlife Sanctuary	Jammu	1,349.80
	Overa Wildlife Sanctuary	Anantnag	3,237.00
	Ramnagar Wildlife Sanctuary	Jammu	1,130.00
	Surinsa, Mansar Wildlife Sanctuary	Udhampur	3,912.00
Himachal Pradesh	Great Himalayan National Park	Kullu	173,600.00
	Bandli Wildlife Sanctuary	Mandi	3,130.00
	Chail Wildlife Sanctuary	Solan	10,855.00
	Daranghat Wildlife Sanctuary	Simla	16,740.00
	Dorlaghat Wildlife Sanctuary	Solan	4,432.00
	Gamgul Siah Behi Wildlife Sanctuary	Chamba	900.75
	Gobind Sagar Wildlife Sanctuary	Bilaspur	10,034.00
	Kalatop Khajjiar Wildlife Sanctuary	Chamba	4,728.00
	Kanawar Wildlife Sanctuary	Kullu	6,070.00
	Khokhan Wildlife Sanctuary	Kullu	1,405.00
	Kias Wildlife Sanctuary	Kullu	—
	Kugti Wildlife Sanctuary	Chamba	11,828.00
	Lippa Asrang Wildlife Sanctuary	Kinaur	10,911.00
	Majathal Wildlife Sanctuary	Simla	9,206.00
	Manali Wildlife Sanctuary	Kullu	3,170.00
	Maina Devi Wildlife Sanctuary	Bilaspur	4,550.00
	Nargu Wildlife Sanctuary	Mandi	27,837.00
	Pong Dam Wildlife Sanctuary	Kangra	30,700.00
	Rakchham Chitkul Wildlife Sanctuary	Kinnaur	13,844.00
	Renuka Wildlife Sanctuary	Sirmour	1,144.00
	Rupi Bhawa Wildlife Sanctuary	Kinnaut	12,486.97
	Suchu Tun Nala Wildlife Sanctuary	Chamba	414.00
	Shikari Devi Wildlife Sanctuary	Mandi	21,350.00
	Simla Water Catchment area Wildlife Sanctuary	Simla	1,025.03
	Shilli Wildlife Sanctuary	Solan	196.70
	Simbal bara Wildlife Sanctuary	Sirmur	1,925.56
	Tirghan Wildlife Sanctuary	Kulu	14,000.00
	Tundah Wildlife Sanctuary	Chamba	6,422.08
Uttar Pradesh	Corbett National Park	Nainital and Pauri Garhwal	52,082.00
	Nanda Devi National Park	Chamoli	63,033.00
	Valley of Flowers National Park	Chamoli	8,750.00
	Govind Wildlife Sanctuary	Uttarkashi	95,312.00
	Kedarnath Wildlife Sanctuary	Chamoli	96,725.51
	Motichur Wildlife Sanctuary	Dehra Dun	—

northwest Himalaya have also been listed under this in order to draw the attention of the public regarding their precarious conditions.

7. Some further suggestions for conservation

Although the general public and Government is aware of the burning problem and

have established a network of protected areas (table 8) some groups such as orchids have not been given due attention. There are some potential areas for orchids development and conservation in north-west region. One such region in temperate belt is the Hindorakhal, a place in Tehri Garhwal district, 8 km beyond Narendranagar on way to Agrakhal. This locality has a *Quercus incana* forest interspersed with *Euphorbia royleana*. There is a profusion of orchid growth in these trees. Though the number of species of orchids is not many the richness in terms of population density of the orchids is very unique. Even the xerophytic plant *E. royleana* supports a host of orchid species indicating the potentiality of the area for orchid growth. The significant species found in this area are *Coelogyne* spp., *Dendrobium bicameratum* Lindl., *D. amoenum* Wall. ex Lindl., *Eria spicata* (D Don) Hand.-Mazz., *Oberonia pachyrachis* Reichb.f. ex Hook.f., *Pholidota articulata* Lindl., *P. griffithii* Hook.f., *Rhynchostylis retusa* Bl., *Thunia alba* (Lindl.) Rechb.f., *Vanda cristata* Lindl. etc. If properly developed this area can form an open orchidarium of northwest Himalaya where all other species could be introduced and multiplied. Similarly the Askot range, Pithoragarh range, Shandev and Didihat, Dafia Dhoora and Kaflani Reserve Forest in Pithoragarh district are also suitable for the establishment of orchid sanctuary. Out of 220 species known so far from hill districts of Uttar Pradesh, 80 species have been recorded from this area. These areas should be declared as Orchid Sanctuaries.

As regards medicinal plants the following one or two suggestions may be considered. Though a ban has been imposed on collection from the wild, a strict enforcement of this law is essential. No doubt the area has a potential for development of medicinal plants. The pharmaceutical industries should be given responsibility of cultivating the medicinal plants for these industries. The biology of several high altitude medicinal plants needs to be properly understood.

Acknowledgement

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Unguiculariella, a new genus of the family Hyaloscyphaceae (Helotiales)

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Abstract. A new genus *Unguiculariella* under the family Hyaloscyphaceae, order Helotiales of the inoperculate Discomycetes is proposed for material collected from Bhutan. The species, *Unguiculariella bhutanica* Thind and R Sharma gen. et. sp. nov., is described and illustrated.

Keywords. *Unguiculariella*; Hyaloscyphaceae; Helotiales: Discomycetes; taxonomy.

1. Introduction

Extensive fungal forays conducted by the senior author and his students in the Himalaya yielded rich collections of higher fungi. A collection made in Bhutan was found to possess certain unique features which can not be accommodated in any known genus of Discomycetes. Therefore, a new genus *Unguiculariella* is proposed with one species, *U. bhutanica* sp. nov. The material has been deposited in the Herbarium of the Botany Department, Panjab University, Chandigarh (PAN). A part of the material is also deposited in the Herbarium, Department of Plant Pathology, Cornell University, Ithaca, New York, USA (CUP).

2. *Unguiculariella* Thind and R Sharma gen. nov.

Apothecia alba, turbinata, parva, villosa. Excipulum ectalum ex textura porrecta, jodo caerulescens, excipulum medullatum ex textura intricata, jodo non caerulescens. Asci octospori, clavati-cylindracei, poro jodo caerulescentc. Ascosporae hyalinae, continuae ad uniseptatae, ellipsoideae. Paraphyses hyalinea, filiformes, apices vitrei, fragiles, jodo colorem caeruleam ducentes. Pili parvi, apices vitrei, jodo colorem caeruleam ducentes, pachydermi, praeter basi.

Apothecia congested, turbinate, white, small, hairy. Asci 8-spored, J+, clavate-cylindric. Ascospores hyaline, non-septate to 1-septate, ellipsoid. Paraphyses filiform, tips glassy, brittle and turning blue in Melzer's reagent. Hairs similar to the paraphyses in the apical part and similarly turning blue in Melzer's reagent, highly thick-walled so as to obliterate the lumen except at the base.

Excipulum differentiated into two zones: (i) ectal textura porrecta, turning blue in Melzer's reagent; and (ii) medullary textura intricata, not turning blue in Melzer's reagent.

Etymology: Refers to the resemblance to the genus *Unguicularia* von Höhnel, in Ann. Mycol. 3: 404, 1905.

The genus is related to *Unguicularia* as erected and diagnosed by von Höhnel (1905)

and accepted by Dennis (1978) and other workers in having glassy hairs with lumen present at the base only but differs in having paraphyses similar to the hairs. Moreover the blue reaction of the ectal excipulum, tops of paraphyses and hairs in Melzer's reagent and turbinate apothecia are unknown for any of the known species of *Unguicularia*. To accommodate these characters, a new genus *Unguiculariella* is erected with a single species, *U. bhutanica*.

Type species: *Unguiculariella bhutanica* Thind and R Sharma *Unguiculariella* Thind and R Sharma has its affinities with glassy-haired genera of Hyaloscyphaceae. The glassy hairs, discussed in detail by Korf and Kohn (1980) and Huhtinen (1987a, b), are of two types depending upon whether their glassiness disappears or is retained in KOH. *Unguiculariella* resembles subgenera *Unguiecularia* Höhn. and *Unguiculariopsis* Rehm as conceived by Korf and Kohn (1980) in retaining hair glassiness in KOH but differs in its glassy hairs turning blue in Melzer's reagent. It also differs from both these genera in glassy paraphyses which like the hairs also retain glassiness in KOH and likewise turn blue in Melzer's reagent. *Unguiculariella* also differs from *Protounguicularia* Raitviir and Galan (1986) by the presence of prominently glassy paraphyses and hairs, which together with ectal excipulum characteristically turn blue in Melzer's reagent and turbinate apothecia.

2.1 *Unguiculariella bhutanica* Thind and R Sharma sp. nov. (figures 1–7). Apothecia maxime gregaria, adpressa, albida, turbinata, basi angusto, cupulata, in sicco ochracea ad pallida fusca, ad 1 mm diametro et ad 1 mm alta, hirsuta. Excipulum ectalum ex textura porrecta, jodo caerulescente, excipulum medullatum ex textura intricata, jodo non caerulescente, hypothecium indistinctum. Asci e uncis nati, clavati-cylindracei, $96–138 \times 10–12\ \mu m$, poro jodo caerulescente, apex rotundatus, pachdermusque. Ascosporae hyalinae, $11–15 \cdot 5 \times 3–5 \cdot 5\ \mu m$, cylindraceae ad subclavatae rectae ad leviter curvatae, continuae ad uniseptatae, guttulatae, irregulariter biseriatae. Paraphyses hyalinae, filiformes, simplices ad ramosae, apices vitrei, ad $2\ \mu m$ latae, pars apicalis conica ad cylindracea ad $18 \times 6\ \mu m$, jodo caerulescente. Pili cylindracei, sursum directi, vitrei, similes parti apicali paraphysium, apex pachydermus, jodo caerulescente.

Holotypus: In petiolo putrescenti angiospermo, in loco humido, Begana, prope Thimphu, Bhutan, August 7, 1981, 24081 (PAN). Leg. R Sharma.

Apothecia gregarious, appressed, white, turbinate, base narrow, turning shallow cupulate, ochraceous to light brown on drying, up to 1 mm in diameter and up to 1 mm in total height, hairy. Asci 8-spored, pore J+, $96–138 \times 10–12\ \mu m$, clavate-cylindric, apex round and thick-walled, up to $1 \cdot 5\ \mu m$ thick, base long, stem-like, arising from croziers. Ascospores hyaline, $11–15 \cdot 5 \times 3–5 \cdot 5\ \mu m$, ellipsoid, short cylindric to sub-clavate, straight to slightly curved, non-septate to 1-septate, guttulate, irregularly biseriate. Paraphyses hyaline, filiform, simple to branched, narrow below, up to $2\ \mu m$ wide, projecting up to $18\ \mu m$ beyond the asci, tips glassy, retaining the glassiness in 2% KOH, conical to short cylindric, up to $18 \times 16\ \mu m$, turning blue in Melzer's reagent. Hairs short, cylindric, up to $14 \times 5\ \mu m$, glassy, brittle, retaining glassiness in 2% KOH, almost similar with the apical part of the paraphyses; tips obtuse, highly thick-walled so as to obliterate the lumen completely
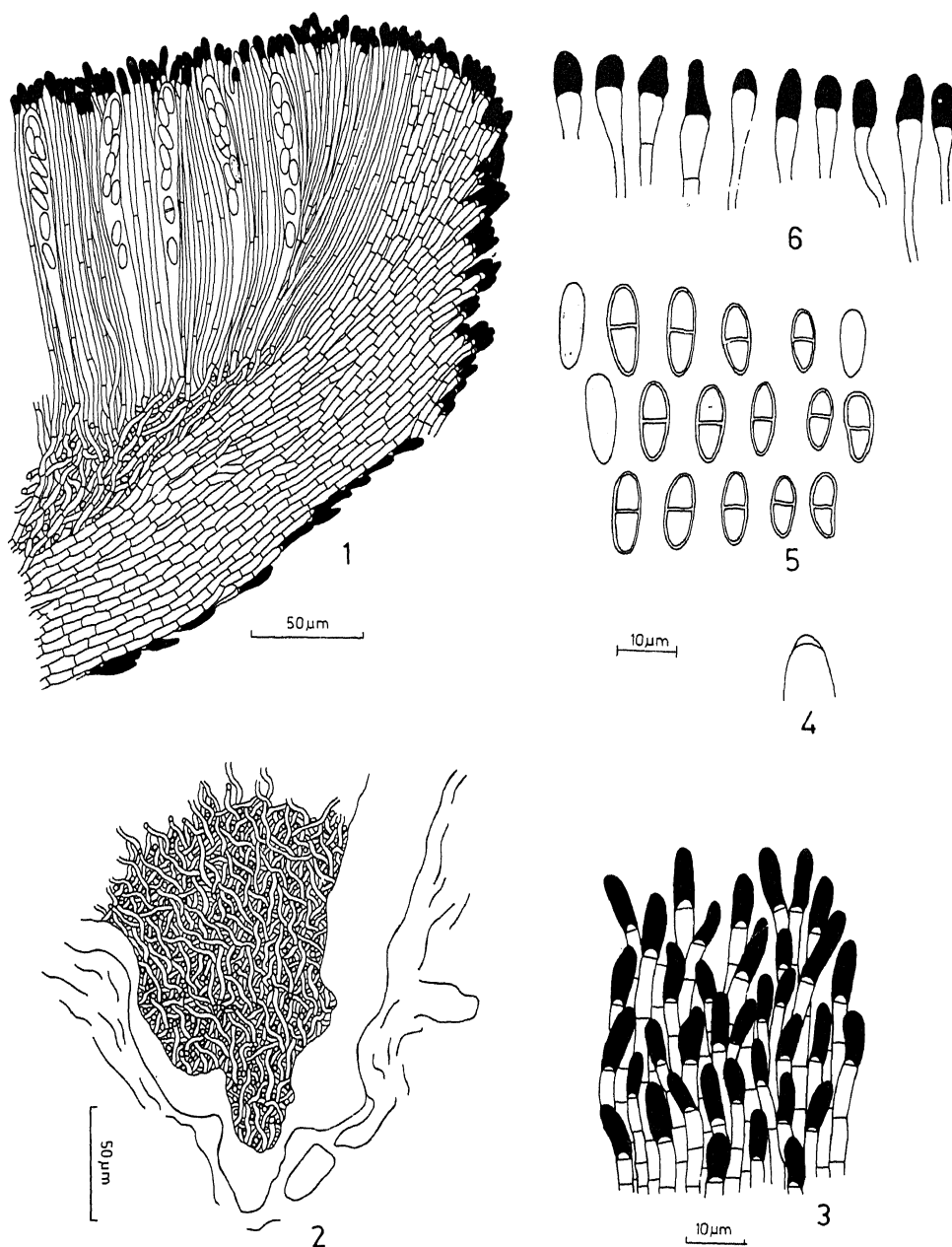
Figure 1–6. *Unguiculariella bhutanica* gen. et sp. nov. **1.** V S apothecium toward margin. **2.** V S apothecium at basal region. **3.** Ectal excipular cells with hairs in surface view. **4.** Ascal tip. **5.** Ascospores. **6.** Paraphyses with tips like the hairs.

Figure 7. Apothecia on decaying leaf stalk of a broad leaved angiosperm.

except at the base, turning blue in Melzer's reagent like the tips of paraphyses, arranged in an ascending manner.

Ectal excipulum textura porrecta, with septa at short intervals nearing textura prismatica, shining, up to 87 μm thick, cells up to 18 × 6 μm, turning blue in Melzer's reagent, outermost cells drawn out into 1-celled hairs; medullary excipulum textura intricata, up to 38 μm thick, cells up to 8 × 4 μm; hypothecium indistinct.

At the base of the apothecium are present thick-walled, shining hyphae forming dense textura intricata, hyphae up to 3·5 μm wide, penetrating the host cells.

Etymology: Refers to the country from where the material was collected.

Collection examined: Holotype: 24081 (PAN), on decaying leaf stalks of some broad leaved angiosperm, in moist place, Begana, Thimphu, Bhutan, August 7, 1981, leg. R Sharma.

The species is characterized by the following features: (i) Glassy brittle hairs are similar to the paraphyses at least in the apical part. (ii) Ectal excipulum, paraphysis apices and hair apices turn blue in Melzer's reagent. (iii) Ectal excipulum of parallel hyphae. (iv) Ascospores large, nonseptate to 1-septate.

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Genus *Dimorphocalyx* Thw. (Euphorbiaceae) in India

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Abstract. A revision of the genus *Dimorphocalyx* Thw. (Euphorbiaceae) for India and adjoining countries is presented. Three species and two varieties are recognised. *Dimorphocalyx beddomei* (Benth.) Airy Shaw is endemic to south India. *Dimorphocalyx lawianus* Hook. f., endemic to south India is reduced to a variety of *Dimorphocalyx glabellus* Thw. *Dimorphocalyx dilipianus* Balakr. and T Chakrab. is reduced to a synonym of *Dimorphocalyx balakrishnanii* T Chakrab. and Premanath, endemic to Andaman Islands. Keys to the taxa, taxonomic descriptions and illustrations are presented.

Keywords. Euphorbiaceae; *Dimorphocalyx*.

1. Introduction

The genus *Dimorphocalyx* was established by Thwaites in 1864 with detailed description, for a plant collected by him in Sri Lanka. The generic name meaning 'calyx of two forms' refer to the accrescent female calyx. Mueller-Argoviensis (1865) reduced *Dimorphocalyx* to a section of *Trigonostemon* Bl., a treatment which he maintained in his account for de Candolle's Prodromus in the following year. However, Bentham (1880) and Pax (1890) reinstated *Dimorphocalyx* to the generic rank. According to Airy Shaw (1973), this Indo-malesian genus, extending to Australia, consists of 12 species. From the Indian subcontinent, 6 species have been described so far. After Thwaites (1864), Hooker (1887) described *D. lawianus* from peninsular India. Another species described in 1878 from Peninsular India by Bentham as *Tritaxis beddomei* was later known to represent a *Dimorphocalyx* as discovered by Airy Shaw. In 1924 Pax and Hoffmann described *D. meeboldii* from Myanmar (Burma). Recently Chakrabarty and Premanath (1983) followed by Balakrishnan and Chakrabarty (1983) discovered two new species from Andamans, namely *D. balakrishnanii* T Chakrab. and Premanath and *D. dilipianus* Balakr. and T Chakrab.

2. Distribution

The species of *Dimorphocalyx* are distributed in Sri Lanka, south India, Andamans, south-east Asia, Indo-China, Hainan, Malaya, west Malesia, New Guinea, Lesser Sunda Is. and north Australia (Queensland). All the species of Indian subcontinent are of restricted distribution. It is satisfying to note that the species of Indian subcontinent still survive in small populations. They are to be found mainly in evergreen or coastal forests (*D. balakrishnanii*) but sometimes also occur in dry regions (in Sri Lanka) and along watercourses (*D. glabellus* var. *glabellus*) or along roadsides (in Sri Lanka) up to about 1100 m altitude. *D. balakrishnanii* is known to grow on sandy soil.

3. Morphology

Amongst the species occurring in Indian subcontinent, the size of leaves, the features of the inflorescences, number of stamens and the characters of the female flowers and fruits are important for specific or varietal delimitation.

There is no chromosome number count available. The pollen grains conform to the typical 'crotonoid' pattern with polygonally arranged clavate sexinous processes (Punt 1962).

Dimorphocalyx Thw., Enum. Pl. Zeyl. 278. 1864; Benth. *apud* Benth. and Hook. f., Gen. Pl. 3: 301. 1880; Hook. f., Fl. Brit. India 5: 403. 1887; Pax in Engler and Prantl, Pflanzenfam. III. 5: 96. 1890; Trimen, Handb. Fl. Ceylon 4: 54. 1898; Brandis, Indian Trees 581. 1906; Cooke, Fl. Pres. Bombay 2: 604. 1906; Bourd., For. Trees Travancore 506. 1908; Talbot, For. Fl. Bombay Pres. and Sind 2: 475. 1911; Pax and Hoffm. in Engler, Pflanzenr. IV. 147. iii: 31. 1911 and in Engler and Harms, Pflanzenfam. ed. 2, 19c: 158. 1931; Haines, Bot. Bihar and Orissa 115. 1921; Gamble, Fl. Pres. Madras 1336. 1925; Airy Shaw in Kew Bull. 20: 412. 1967 and 36: 286. 1981; Whitmore, Tree Fl. Malaya 2: 86. 1973.
Trigonostemon Bl. sect. *Dimorphocalyx* (Thw.) Muell.-Arg. in Linnaea 34: 212. 1865 and in DC., Prodr. 15(2): 1105. 1866.

Type species: *D. glabellus* Thw.

Dioecious or rarely monoecious shrubs or small trees, very rarely scandent shurbs, nearly glabrous. Leaves simple, alternate, short-petioled, usually elliptic to oblong or obovate or ovate, glandular-denticulate to crenulate to entire; thinly chartaceous to thinly coriaceous, penninerved; midrib flat above, raised beneath; lateral nerves slender, arcuate or somewhat straight, anastomosing near margins and joining the superadjacents forming loops; tertiary nerves reticulate; stipules triangular to deltoid, short, deciduous. Inflorescences unisexual or rarely bisexual, cymose, axillary and terminal, solitary to 2–3-flowered (umbellate) to shortly racemiform or thyrsoid, often tending towards dichasial branching. Male flowers bracteate, pedicellate; calyx cupular, 5-lobed, imbricate; petals 5, white, imbricate; disk of 5 free glands; stamens biseriate, 8–16, the outer usually free or sometimes partially and shortly connate, the inner united into a column; anthers 2-celled, adnate to a broad connective, dorsifixed or basifixed (in the same flower), longitudinally dehiscent; pistillode O. Female flowers bracteate, pedicellate; sepals 5, free or shortly connate, of various shapes, imbricate, often glandular-pitted or emarginate at apex, accrescent in fruit; petals white, oblong, spathulate, oblong to obovate, imbricate, caducous; disk glands connate in a ring or shortly cupular-annular; staminodes O; ovary subglobose or trigonous-ovoid, 3-locular (locules 1-ovuled; ovules anatropous), glabrous or pubescent; styles 3, connate at base or free, bifid, erect or spreading. Capsules tricoccous, globose or subglobose, evanescently scattered pubescent or glabrous, woody, splitting into bivalved parts leaving central column; seeds broadly oblong-ellipsoid or obovoid, trigonous with a broad convex back; endosperm fleshy; cotyledons flat, broad.

The genus is placed under the tribe *Codiaeae* by Webster (1975) in the subfamily *Crotonoideae* along with another 18 genera. As per the circumscription of the genus,

it is a natural taxon, well characterized by the dioecious habit (mostly), usually short-petioled glandular-serrate to entire penninerved leaves, the short, unisexual (bisexual only in *D. balakrishnanii*) cymose inflorescences, the outer whorl of free stamens, the inner whorl of united stamens, the caducous white petals and the female calyces frequently being accrescent in fruit. The genus can be distinguished from the related *Trigonostemon* Bl. by the leaves being not or obscurely trinerved (triplinerved) at base and glandular-denticulate to entire or occasionally remotely crenate, the dioecious flowers, the white petals, 7–16 stamens (the outer more or less free, the inner united in a column) and the accrescent female calyx. From *Ostodes* Bl., the genus differs in its not or obscurely trinerved leaves with glandular-dentate to entire margins, the glabrous filaments of stamens (except *D. balakrishnanii* having basally pilose filaments) with the inner whorl united and the accrescent female calyx. None of the species is reported to have any particular economic or medicinal use.

Key to the species

1a. Plants monoecious; inflorescences bisexual (occurring in Andaman Islands) . . . . **1. D. balakrishnanii**
  b. Plants mostly dioecious (occasionally monoecious); inflorescences unisexual (occurring in south India and Sri Lanka). 2
2a. Male and female inflorescences somewhat dichasial; stamens 8; ovary glabrous; styles 2–3 mm long, free; capsules glabrous . . . .**2. D. beddomei**
  b. Male and female inflorescences not dichasial; stamens 10–16; ovary pubescent; styles 3–7 mm long, connate below into a column; capsules evanescently pubescent . . . .**3. D. glabellus**

1. D. balakrishnanii T Chakrab. and Premanath in J. Econ. Tax. Bot. **4**: 1013, f. 1. 1983. *Types*: India, Andaman Is., Havelock Is., *ca* 40 m alt., 15 May 1974, *Ansari 1368A* (CAL-holotype); *ibid. Ansari 1368B-1368C* (PBL-isotypes); south Andamans Is., Goplakabang, 1890, *King's Coll.* s.n., Herb. Acc. No. 412226 (CAL-paratype); *sine loc. exact.*, 1900, *Prain's Coll.* 60 (CAL-paratype).

D. dilipianus Balakr. and T Chakrab. in J. Econ. Tax. Bot. **4**: 1017, f. 1. 1983, synon. nov. *Types*: India, Andaman Is., south Andamans, Corbyn's Cove, *ca* 20 m alt., 10 Oct. 1973, *N G Nair 498A* (CAL-holotype); *ibid.*, *N G Nair 498B–498 E* (PBL-isotypes).

Scandent shrubs or trees, *ca* 6 m tall, nearly glabrous; branchlets greyish or brown, terete, smooth, 1·5–5 mm thick, often angular when young. Leaves elliptic to oblong or ovate-oblong to ovate-elliptic, 6–24 cm long, 3–11·5 cm wide, acute, cuneate, obtuse or rounded at base, entire or sometimes shallowly crenate-dentate at margins, acuminate (acumen 10–20 mm long, acute or obtuse) to acute or occasionally rounded at apex, thinly chartaceous to thinly coriaceous, brown or blackish above when dry, pale brown or blackish-brown beneath; midrib flat above, raised beneath; lateral nerves slender, 6–10 pairs, faint to prominent above, distinct beneath, arcuate or often more or less straight, anastomosing and upturning near margins, joining the superadjacents forming loops; tertiary nerves obscure to

prominent above, faint to distinct beneath, reticulate; petioles 4–13 mm long, 1–3 mm thick, channelled above; stipules triangular to deltoid, 1–2 mm long, deciduous. Inflorescences terminal and axillary, few-flowered, androgynoeceous or occasionally bearing 1–2 females (solitary or umbellate), peduncled (2–5 cm long) with inconspicuous rachis (umbellate) or epeduncled with 5–30 mm long rachis (racemiform); bracts deltoid or triangular to linear-subulate, 1–6 mm long. Male flowers: pedicels 2·5–3 mm long, sparsely puberulous to glabrous; calyx cupular, shallowly to deeply 5–lobed, sparsely fulvous-puberulous to glabrous, 3–4 mm long; lobes broadly triangular to deltoid or orbicular or ovate-oblong to elliptic, 1–3 mm long, minutely ciliate at margins; petals 5, narrowly elliptic to oblong or spathulate or obovate to oblanceolate, 7–10 mm long, 2–5 mm broad; disk glands 5, transversely oblong; stamens 4–5 + 5–6, the outer free, the inner monadelphous; outer filaments 2·5–4 mm long; inner united column 4–6 mm long (basal connate portion 2–3 mm long), pilose near base (recalling *Ostodes* Bl.); anthers ellipsoid, oblong, ovoid or orbicular, 0·8–1·2 mm long. Female flowers: pedicels 2–5 mm long, fulvous-puberulous to often subglabrous; sepals 5, unequal, elliptic to oblong or oblong-lanceolate or narrowly obovate-oblong to oblanceolate, acute to obtuse, softly fulvous-puberulous outside (at least at the base) and inside, often becoming glabrescent, accrescent in fruit; petals 5, narrowly oblong to oblanceolate, 10–18 mm long, 3–5 mm broad, caducous; disk glands connate in a ring; ovary subglobose, 2–2·8 mm long, 2·8–3 mm diam., densely ochraceous- or tawny-puberulous or tomentellous, 3-lobed; styles 3, 7–8·5 mm long, connate below into a column (0·5 −) 2–3 mm long, densely puberulous, bifid above. Capsules tricoccous, subglobose, *ca* 15 mm long, 15–17 mm diam., adpressed ochraceous- or fulvous-puberulous, black or black-brown when dry; fruiting pedicels 6–12 mm long; sepals 8–50 mm long, 3–20 mm broad; seeds broadly oblong-ellipsoid, *ca* 10 mm long, *ca* 6 mm diam., smooth, brown (figure 1).

Flowering and fruiting: May-Oct. (sometimes Feb.).

Specimens examined: India. *Andaman Is.: sine loc. exact.*, 1884, *King's Coll.* 139 (CAL); *ibid.*, Oct. 1900, *Prain's Coll.* 29 (CAL); *ibid.*, 1900, *Prain's Coll.* 5 (CAL). *South Andaman Is.*: Cadelgunj hill jungle, no date, *King s.n.*, Herb. Acc. No. 412230 (CAL); Corbyn's Cove, 13 Feb. 1892, *King's Coll. s.n.*, Herb. Acc. No. 412222 (CAL).
Distribution: South Andamans (south Andaman Is., Havelock Is.) — Endemic.

Ecology: Very rare in inland evergreen forests or coastal forests on sandy soil at low altitudes.

A puzzling species, perhaps somewhat isolated taxonomically, differing from all of its congeners by the androgynoeceous inflorescences. It is evident from the additional old collections in CAL (as cited above) that except for the scandent habit, the other supposed differences of *D. dilipianus* from *D. balakrishnanii*, i.e. the relatively stiffer leaves with rounded base, larger rachis of inflorescences, larger female petals and the larger fruiting sepals do not hold good. It will have to be ascertained from further collections that whether the scandent habit of *D. dilipianus* is a constant character, as in such case this taxon may deserve a varietal status. The scandent habit is, however, also known in one example of *D. glabellus* var. *glabellus*.
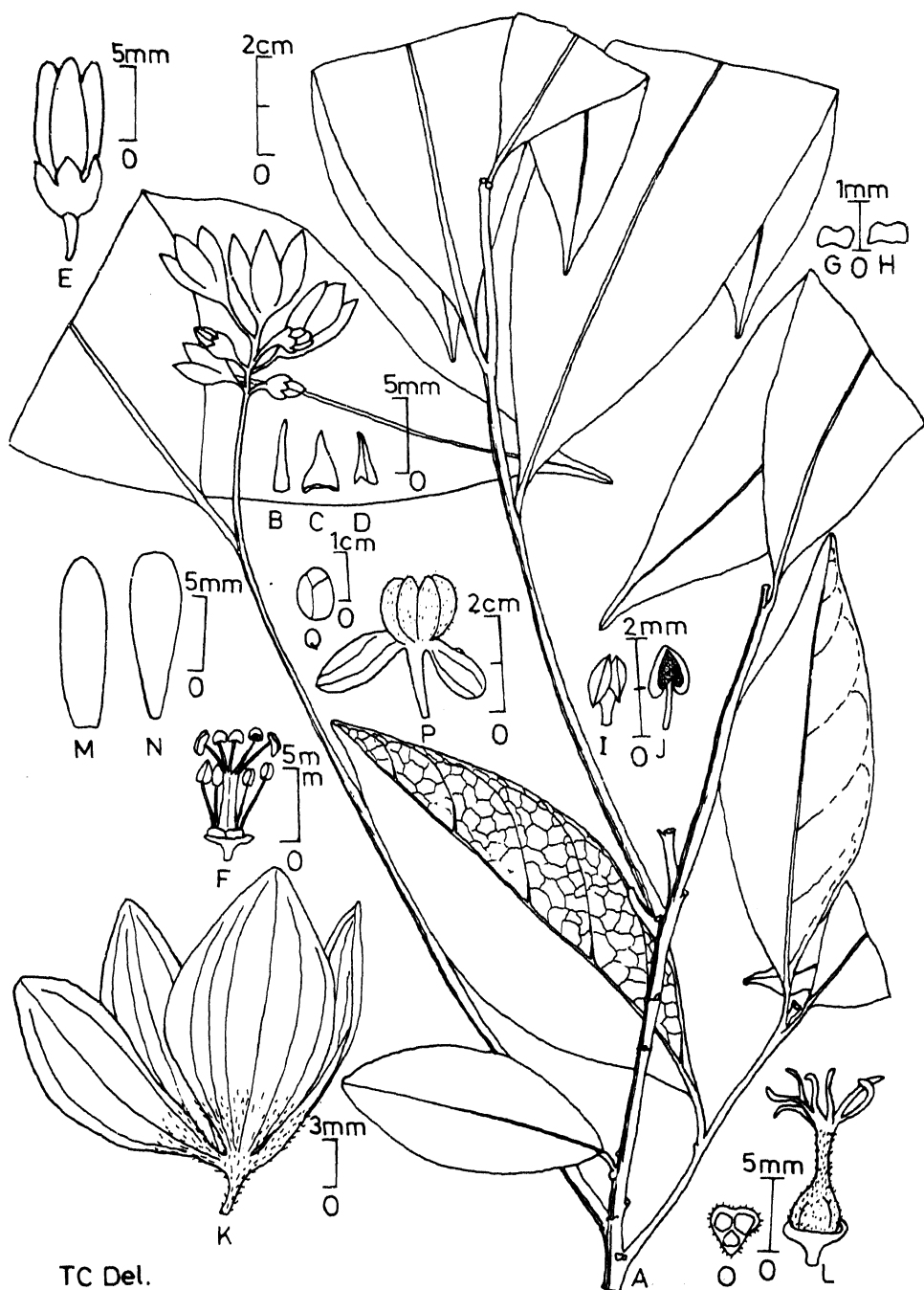
Figure 1. *D. balakrishnanii.* **A.** Habit. **B–D.** Bracts. **E–J.** Male. **E.** Flower. **F.** Androecium. **G–H.** Disk glands. **I–J.** Anthers. **K–O.** Female. **K.** Flower. **L.** Ovary with disk. **M–N.** Petals. **O.** TS of ovary. **P.** Fruit. **Q.** Seed.
[A–O. *King's Coll.* 139 (CAL), P, Q. *King's Coll.* s.n. (CAL)]

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Trigonostemon beddomei (Benth.) Balakr. in Bull. Bot. Surv. India 10: 245. 1969 (1968).

Dimorphocalyx glabellus sec. Nair and Bhargavan in Indian J. For. 4: 158. 1981, *pro parte, tantum quoad Bhargavan* 47484, *non* Thw. 1864.

Dioecious or sometimes monoecious shrubs or trees, up to 4 m tall, glabrous; branchlets brown or blackish, terete, smooth, 1·5–4 mm thick. Leaves ellitpic to oblong or narrowly so to elliptic-lanceolate, 5–18 (–22) cm long, 2–6 (–7·5) cm broad, rounded, obtuse, acute to cuneate (often unequal-sided) at base, minutely glandular-toothed or shallowly sinulate to subentire at margins, acutely to obtusely acuminate (acumen 5–20 mm long) to subacute at apex, thinly coriaceous, greenish or pale brown or blackish above when dry, greenish to pale brown beneath; midrib flat above, raised beneath; lateral nerves 7–12 pairs (the first pair shorter and weaker than the subsequent pairs), faint above, faint to prominent beneath, arcuate or somewhat straight, anastomosing near margins and joining the superadjacents forming loops; tertiary nerves obscure to faint above, faint to prominent beneath, reticulate; petioles 5–15 mm long, 1–2 mm thick, shallowly channelled above. Male inflorescences axillary (often ramiflorous?), more or less dichasially branched, up to 4·5 cm long, shortly pedunculate (peduncle *ca* 1 cm long); bracts deltoid, 0·7–1 mm long, 1–1·3 mm broad, puberulous at margins. Flowers 4–6 mm across, white; pedicels *ca* 1·2 mm long, *ca* 0·7 mm thick; calyx cupular, shallowly 5-lobed, *ca* 2 mm high, *ca* 3 mm diam.; lobes suborbicular, *ca* 1 mm long, obtuse; petals 5, narrowly oblong, 5–6 mm long, 1·6–3 mm broad, often emarginate; disk glands 5, triangular-oblong to linear-subulate, *ca* 1 mm long, 0·2–0·3 mm broad; stamens 5 + 3, the outer free to partially (basally) connate, the inner monadelphous; outer filaments *ca* 4 mm long; inner united column *ca* 5 mm long; anthers broadly oblong or ovoid, *ca* 1 mm long. Female inflorescences terminal, also terminating the short lateral fertile shoots bearing 2–3 immature leaves, somewhat dichasially branched, few-flowered, up to 5 cm long, often solitary or 2–3-flowered (umbellate), shortly pedunculate (peduncle *ca* 1 cm long) to epedunculate; bracts deltoid, 1–1·5 mm long, 1·5–1·8 mm broad, puberulous at margins. Flowers 9–11 mm across; pedicels 3–4 mm long, 1–1·5 mm thick towards apex, 0·5–0·7 mm thick towards base; sepals 5, oblong to oblong-elliptic or obovate-oblong, 5–6 mm long, 3·5–4 mm broad, shortly connate, often emarginate at apex, accrescent; petals 5, oblong to spathulate, 5–6 mm long, 2·5–3 mm broad; disk glands connate in an annular ring; ovary globose, *ca* 2 mm diam., 3-lobed, glabrous; styles 3, 2–3 mm long, each bifid; stigmas simple. Capsules subglobose, depressed, tricoccous, 11–13 mm long, 16–18 mm diam.; fruiting sepals up to 15 mm long, up to 13 mm broad; seeds said to be obtusely trigonous, *ca* 8 mm long, *ca* 7 mm diam., mottled, marbled, with crustaceous testa (figure 2).

Flowering and fruiting: May-Oct.

Specimens examined: India. *Kerala*. Trivandrum dist., Forest near Bonaccord
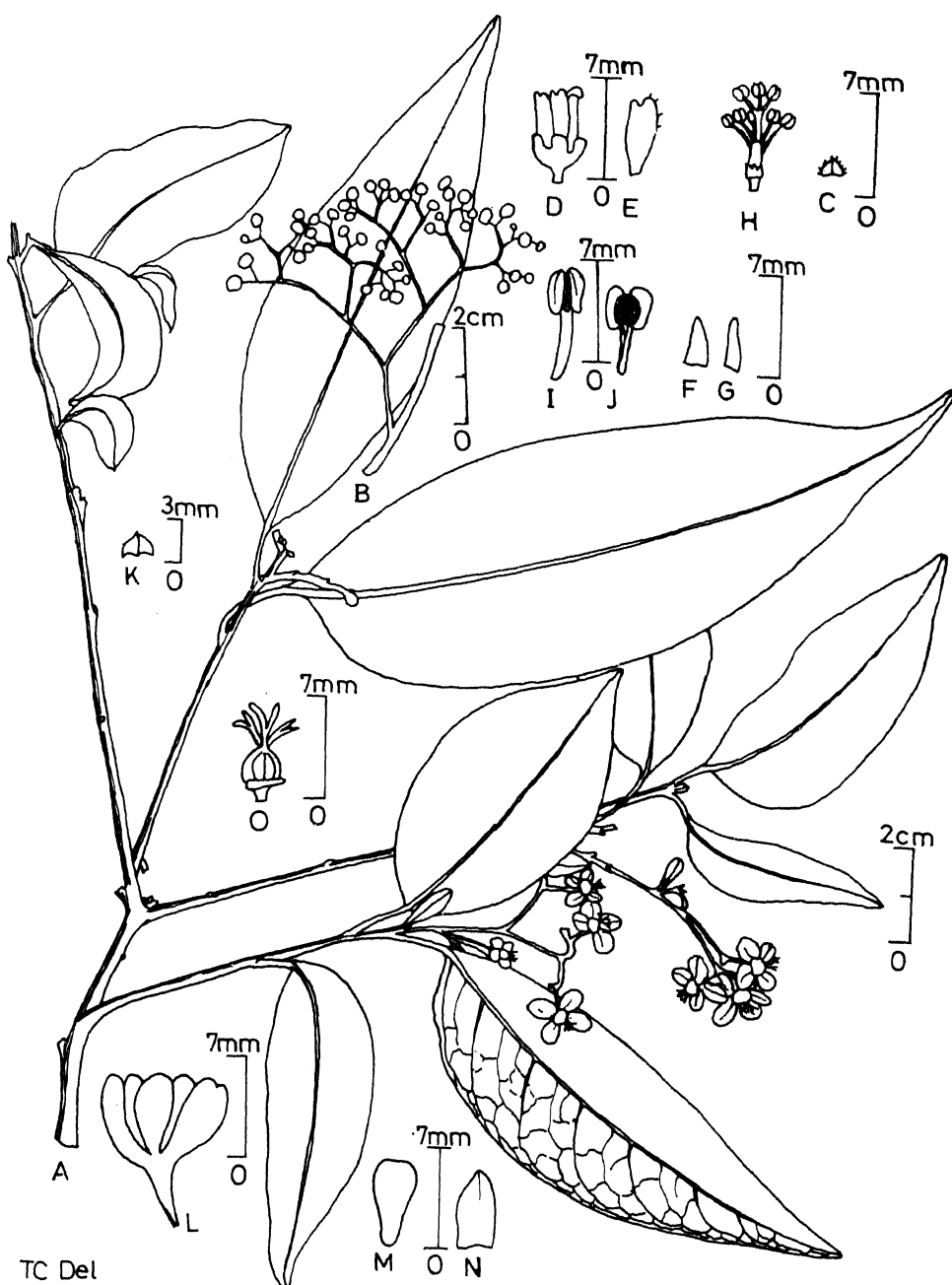
Figure 2. *D. beddomei.* **A.** Habit with female flowers. **B.** Male inflorescence. **C–J**: Male. **C.** Bract. **D.** Flower. **E.** Petal. **F, G.** Disk glands. **H.** Androecium. **I, J.** Stamens. **K–O.** Female. **K.** Bract. **L.** Flower. **M, N.** Petals. **O.** Ovary with disk.
[**A, K–O.** *Bhargavan* 47489 (CAL), **B.** *Beddome* 37 (K), **C–J.** *Joseph* 44509 (CAL)].

estate, 2 Oct. 1973, *Joseph* 44509 (CAL, MH); Koviltheri forest, Bonaccord estate, 24 Aug. 1975, *Joseph* 46510 (CAL, MH). *Tamil Nadu.* Tirunelveli Dist., Valayar forest, 13 July 1976, *Bhargavan* 47484 (CAL, MH); Kannikatti to Agastyamalai, 25

Apr. 1972, *Henry* 8425 (MH); Kannikatti to Kalivayalpil, 23 Aug. 1963, *Henry* 16994 (MH).

Distribution: South India (Kerala, Tamil Nadu)-Endemic.

Ecology: Evidently rare in evergreen forests at 850–1000 m altitude.

Distinguished from *D. glabellus* by the somewhat dichasial arrangement of the inflorescences, the fewer stamens, the glabrous ovary (and fruits) and the much shorter free styles.

There has been some discrepancy as to the correct spelling of the specific epithet of the species, which commemorates R H Beddome. Bentham (1878) used the spelling *beddomi* for the taxon, and was followed by Airy Shaw (1969, 1972), while all other authors used the spelling *beddomei*. In a personal communication (dated 17 June 1983), late Mr H K Airy Shaw clarified the situation. He wrote: 'Expert opinion here (i.e. at Kew) considers that 'beddomei' is the spelling to be adopted, with (Benth.) still given as the original author'.

Bhargavan collected the long-unknown female plant in 1976 from near the type locality, but determined the material to be *D. glabellus* and subsequently Nair and Bhargavan (1981) included the specimen in their account on the rediscovery of *D. glabellus*. Finally Henry *et al* (1982) came across the female plant material of *D. beddomei* and emended the description of the species. They also remarked that the plants of *D. beddomei* are monoecious, and not dioecious as described in earlier literature. This statement is however not fully correct because of the fact that dioecism is the usual feature in *Dimorphocalyx* although monoecism may occasionally occur, and that the type of *D. beddomei* itself was obtained from a male plant. In addition, the material *Bhargavan* 47484 also represents a female plant specimen. The other specimens as cited above also bear male inflorescences only.

3.   **D. glabellus** Thw., Enum. Pl. Zeyl. 278. 1864; Hook. f., Fl. Brit. India 5: 403. 1887; Trimen, Handb. Fl. Ceylon 4: 54, fig. 84. 1898; Woodrow in J. Bombay Nat. Hist. Soc. 12: 371. 1899; Brandis, Indian Trees 581. 1906; Bourd., For. Trees Travancore 506. 1908; Pax and Hoffm. in Engler, Pflanzenr. IV. 147. iii: 32, figure 8. 1911; Haines, Bot. Bihar and Orissa 2: 115. 1921; Gamble, Fl. Pres. Madras 1337. 1925; Nair and Bhargavan in Indian J. For. 4: 158. 1981 (sphalm. *"glabellatus"*) *pro majore parte*, excl. *Bhargavan* 47484. *Types*: Sri Lanka, Central Prov., no date; *Thwaites* CP 1046 (2167) (CAL: lectotype chosen herein); *ibid.*, *Thwaites* CP 1046 (2167) (K: photo, G-DC: microfiche-2 sheets-isolectotypes).
Croton glabellus Heyne *apud*. Wall. Cat. No. 8012 A and B. 1847, *nomen nudum*.
Croton ramiflorus Graham, Cat. Pl. Bombay 182. 1839, *e descr. Type*: India, Maharashtra, *Graham* (n.v.).
Trigonostemon lawianus sec. Muell.-Arg. in Linnaea 34: 212. 1865 et in DC., Prodr. 15(2): 1105. 1866 (non *Croton lawianus* Nimmo 1839).
Dimorphocalyx lawianus Hook. f., Fl. Brit. India 5: 404. 1887, synon. nov. *Types*: India, Malabar, Concan, etc., no date, *Stocks, Law*, etc. s.n. (MH-lectotype chosen herein, K: photo!).

Trees or sometimes scandent shrubs, dioecious or sometimes monoecious, 3–6 m tall, nearly glabrous; bark smooth, grey to brown; branchlets whitish, grey, brown

or reddish brown, 1–5 mm thick, smooth, initially angled, finally terete. Leaves narrowly to broadly elliptic, oblong to obovate and oblanceolate, sometimes ovate to ovate-lanceolate, occasionally suborbicular, 1·5–1·9 cm long, 0·5–8 cm wide, rounded, obtuse to acute or often cuneate at base, shallowly glandular-crenulate (particularly in young leaves), crenate-serrate to entire at margins, bluntly acuminate (acumen 5–30 mm long), to acute or often obtuse to rounded or cuspidate at apex, thinly coriaceous to chartaceous, remaining green or turning brownish, black or blackish carmine above when dry, paler beneath; midrib flat above, raised beneath; lateral nerves 6–14 pairs, very slender, obscure to prominent above, faint to distinct beneath, arcuate or somewhat straight, anastomosing near the margin and joining superadjacents forming loops; tertiary nerves obscure to prominent, reticulate or often tending to branch into nerves of higher order (transverse-ramified); petioles 2–20 mm long, 0·5–3 mm thick, sulcate or shallowly channelled above. Male inflorescences axillary, solitary or umbellately 2–3-flowered or in few-branched cymes, rarely narrowly thyrsiform, up to 4 cm long, often with up to 5 mm long peduncles; bracts oblong, triangular to deltoid, 1–3 mm long, 1–3·5 mm wide. Flowers: pedicels 2–6 mm long, 0·5–1·2 mm thick; calyx cupular, shortly 5-lobed, 2·5–5 mm high, 3–6 mm diam.; lobes deltoid, triangular to orbicular, 0·5–1·5 mm long; petals 5, oblong, elliptic, obovate to spathulate-obovate, rounded at apex, 5–6 mm long, 2–2·5 mm wide; disk glands 5, transversely oblong to obcordate, 0·3–1 mm long, 0·5–1 mm diam., yellow puberulous at apex or glabrous; stamens $5-6+6-10$ (i.e. $11-16$), the outer free to shortly and partially connate, the inner ones united into a column; outer filaments 1–4 mm long, inner united column 3–7 mm long; anthers ovoid, oblong, ellipsoid to orbicular, 0·5–1·2 mm long. Female inflorescences axillary and terminal, also terminating short lateral fertile shoots bearing immature leaves, solitary or 1-few-flowered, umbellate or up to 4 cm long racemes, often with 0·5–4 mm or 1–3 cm long peduncles; bracts triangular, deltoid, oblong to linear, 1·5–7 mm long, 0·5–3 mm wide. Flowers: pedicels 3–10 mm long, 1–2 mm thick towards base, 2–3 mm thick towards apex, evanescently and densely ochraceous-puberulous to tomentellous; sepals 4–5, subequal, free, spathulate, elliptic-oblong to obovate or narrowly ovate to lanceolate, obtuse or rounded or glandular-pitted or emarginate at apex, evanescently depressed ochraceous-tomentellous towards base, often accrescent; petals 4–5, elliptic, broadly oblong to obovate or suborbicular, 5–11 mm long, 2·5–7 mm wide, caducous; disk shortly cupular-annular or forming a ring, entire or crenate, 0·6–1 mm high, 3–4·5 mm diam.; ovary subglobose or trigonous-ovoid, 2–4 mm long, 2·5–5 mm diam., 3-lobed or unlobed, ochraceous or tawny-tomentellous or hirsute; styles 3, 3–7 mm long, mostly shortly connate below into 0·5–3 mm long puberulous column; lobes bifid above, erect or spreading. Capsules subglobose, tricoccous, 7–15 mm long, 10–18 mm diam., blackish or dark brownish when dry, densely and evanescently scattered appressed-hirsute or ochraceous-puberulous; pedicels up to 12 mm long; accrescent sepals 5–33 mm long, 3–18 mm wide; seeds oblong, ellipsoid, obovoid or subtrigonous and dorsally convex, 5–10 mm long, *ca* 8 mm diam., brown, marbled.

Key to the varieties

1a. Leaves small, averaging 1·5–12 (−15) cm long, up to 4(−5) cm wide; stamens

10–11; capsules 7–10 mm long, 10–12 mm thick; fruiting sepals relatively smaller, 5–12(– 16) by 3–10 mm, equal or subequal . . . . . . **3a. var. glabellus**
b. Leaves larger, averageing, (4·5 –) 6·5–19 cm long, up to 6 (– 7) cm wide; stamens 11–16; capsules 12–15 mm long, 15–18 mm thick; fruiting sepals relatively larger, 12–32 by 4–18 mm, subequal or unequal . . . . **3b. var. lawianus**

3a. var. glabellus

Trees, once noted to be a scandent shrub, dioecious, sometimes monoecious. Leaves 1·5–12(–15) cm long, up to 4(– 5) cm wide; acumen at apex 5–10 mm long; lateral nerves 6–12 pairs; petioles 2–15 mm long, 0·5–2 mm thick. Male inflorescences axillary and terminal, few-flowered, subumbellate with up to 5 mm long peduncles. Flowers pedicels 2–3 mm long, 0·5–0·8 mm thick; calyx 2·5–3 mm high, 3–4 mm diam.; petals oblong, rounded at apex, 5–6 mm long, 2–2·5 mm wide; disk glands 0·3–0·5 mm long, 0·5–0·8 mm diam.; stamens 5 + 5 (– 6); outer filaments 1–4 mm long, inner united column 3–4 mm long. Female inflorescences axillary and terminal, 1-few-flowered umbels with up to 4 mm long peduncles; bracts deltoid, triangular to narrowly oblong, 1·5–4 mm long, 0·5–3 mm wide. Flowers: sepals (4 –) 5, obovate to oblanceolate elliptic to lanceolate or oblong; petals 5, elliptic, oblong to obovate, 5–11 mm long, 2·5–6 mm wide; styles 3–6 mm long, united into 0·5–2 mm long column, free and bifid above. Capsules 7–10 mm long, 10–12 mm diam., dark brownish when dry, yellow or ochraceous puberulous; pedicels up to 12 mm long; accrescent sepals 5–16 mm long, 3–10 mm wide; seeds ellipsoid, 5–8 mm long, 3–6 mm wide (figure 3).

Flowering and fruiting: Jan.-Dec.

Local names: Sinhalese: *Wellewenne-gas*; Tamil: *Kalpottan, Velleipula.*

Specimens examined: India. Peninsular India, *sine loc. exact.*, no date, *Heyne in Herb. Wallich* 7750 (K-WALL: microfiche, p.p., upper twig only); *ibid.*, no date, *Heyne in Herb. Wallich* 8010, 8012 A (CAL, K-WALL: microfiche); *ibid., no date, Wallich (Heyne?)* 8012B (K-WALL: microfiche); *ibid.* no date, *Wight* 70 (CAL). *Andhra Pradesh*: Visakhapatnam, Dolphin's nose, Feb. 1885, *Gamble* 16105 (CAL, MH). *Kerala*: Purapur, 5 May 1904, *Rama Rao* 95 (CAL). *Orissa*: *sine loc. exact.*, no date, *Haines* s.n. Herb. Reg. No. 18525 (DD); Ravine in Angul, Mar. 1917, *Haines* 4042 (CAL). *Maharashtra*: Pune Dist., Ambavana-Mulshi Taluk, Wagja forest, on way to Mangaon, 8 Sept. 1964, *B V Reddy* 99129 (CAL); Bhimasankar, 15 Dec. 1907, *Talbot* 5013 (BSI). *Tamil Nadu*: Coimbatore Dist., Karianshola, 2 July 1976, *Chandrabose* 47251 (CAL). Sri Lanka. Trincomale, 20 Mar. 1922, no collector s.n., Herb. Reg. No. 35751 (DD); Trincomale to Anuradhapura, 25 Mar. 1956, *D. Chatterjee* 536 (CAL); Sabaragamuwa Prov., Ratnapura Dist., *ca* 10 mile southeast of Godakewela on the Pelimadulla-Humbantala Road, 24 Nov. 1974, *Davidse and Sumithraarachi* 8787 (CAL).

Distribution: **South India and Sri Lanka.**

Ecology: - Sri Lanka: Common in dry regions on sheer bare rock outcrops with
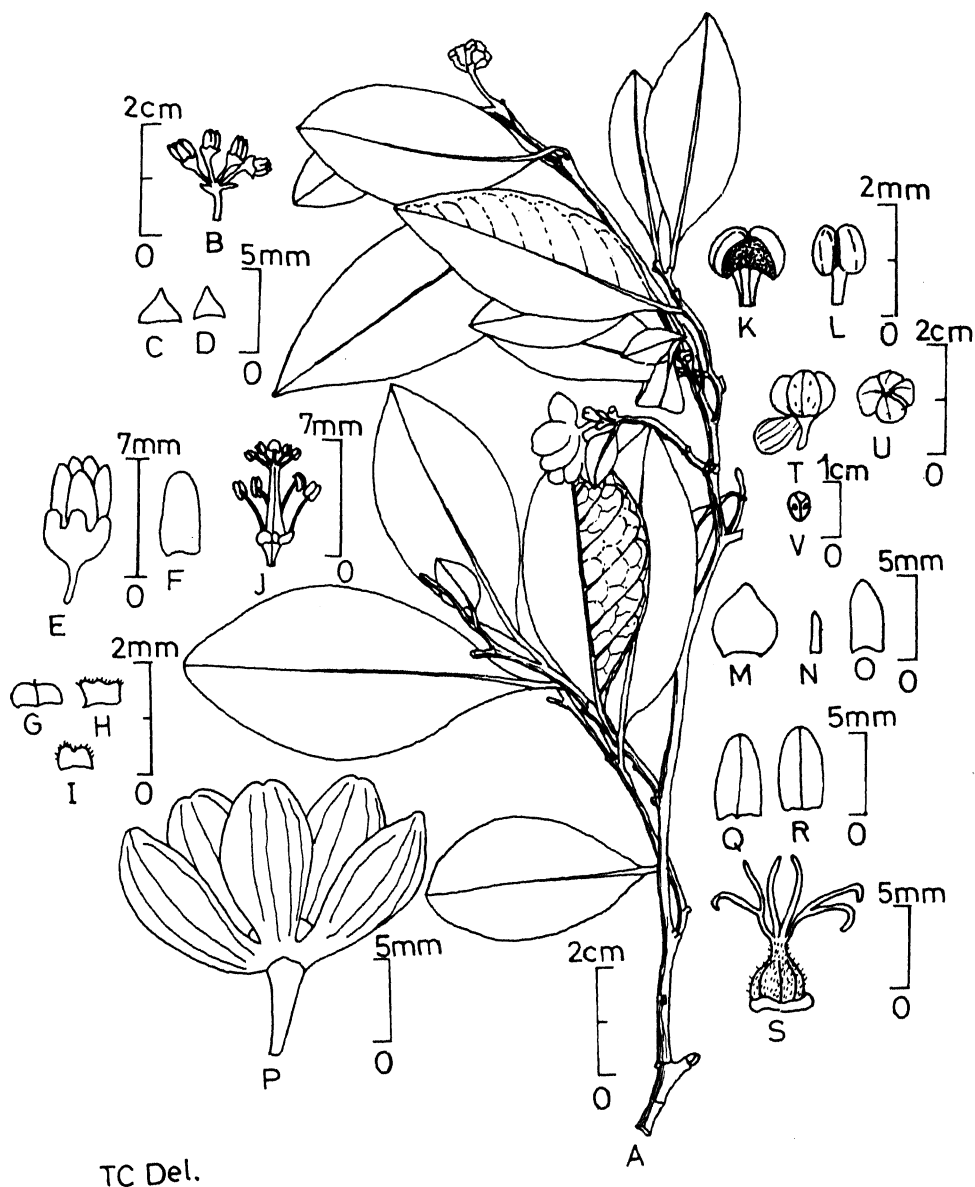
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Figure 3. *D. glabellus* Thw. var. *glabellus.* **A.** Habit. **B.** Male inflorescence. **C–L.** Male. **C, D.** Bracts. **E.** Flower. **F.** Petal. **G–J.** Disk glands. **J.** Androecium. **K–L.** Anthers. **M–S.** Female. **M–O.** Bracts. **P.** Flower. **Q, R.** Petals. **S.** Ovary with disk. **T, U.** Fruits. **V.** Seed. [**A, P–S.** *Chatterjee 536* (CAL). **B–L, T–V.** *Davidse and Sumithraarachi 8787* (CAL). **M–O.** *Haines 2507* (MH)].

sparsely wooded dry watercoarse and along roadsides, up to *ca* 300 m altitude. South India: Scarce in evergreen forests, up to 900 m altitude.

Nair and Bhargavan (1981) rediscovered the plant in 1981 after a gap of 91 years. However, typical *D. glabellus* occurs in Sri Lanka (from where the genus and its type species were originally described). A definite break in the population occurs in

Peninsular India where all parts of the plant tend to enlarge and thereby the plant itself sometimes tend to merge with *D. lawianus*. In such cases, the slightly smaller capsule (of *D. glabellus*) is the only reliable distinction. It is therefore necessary to recognise *D. lawianus* only as a variety of *D. glabellus* as below.

3b. var. lawianus (Hook. f.) T Chakrab. and Balakr. **comb. et stat. nov.** *Dimorphocalyx lawianus* Hook. f., Fl. Brit. India 5: 404. 1887; Woodrow in J. Bombay Nat. Hist. Soc. 12: 371. 1899; Brandis, Indian Trees 581. 1906; Cooke, Fl. Pres. Bombay 2: 604. 1906; Bourd., For. Trees Travancore 508. 1908; Talbot, For. Fl. Bombay Pres. and Sind. 2: 475. 1911; Pax and Hoffm. in Engler, Pflanzenr. IV, 147. iii: 31. 1911; Fischer in Rec. Bot. Surv. India 9(1): 164. 1921; Gamble, Fl. Pres. Madras 1337. 1925. *Types*: India. Malabar, Concan, etc., no date, *Stocks, Law etc.* s.n. (MH-lectotype chosen herein, K: photo! -BSI, Neg. No. 8613 B); *ibid. Stocks, Law*, etc. s.n. (CAL, G-DC: microfiche! -iso-lectotype); Tamil Nadu, Anamallays, 1964, *Beddome* s.n. -female (MH); Concan, no date, *Law* s.n. (K: photo). *Trigonostemon lawianus sec.* Muell.-Arg. in Linnaea 34: 212. 1865 and in DC., Prodr. 15(2): 1105. 1866 (non *Croton lawianus* Nimmo 1839), *pro parte, tantum quoad* Stocks in Hook. f. and Thoms. herb. Ind. Or.; Bedd., Fl. Sylv. South India t. 272 (excl. fig. 10–12). 1872, excl. syn. and For. Man. 212. 1873. *D. glabellus sec.* Bedd. in Trans. Linn. Soc. London 25: 225, t. 26 (excl. fig. 10–12). 1866, *non* Thw. 1864.

Trees, dioecious. Leaves (4·5 −) 6·5–19 cm long, (2 −) 3 8 cm wide; acumen at apex 5–30 mm long; lateral nerves 6–14 pairs; petioles 3 20 mm long, 1·2 3 mm thick. Male inflorescences axillary, solitary or 2 3-flowered umbels or few-branched cymes or narrowly thyrsiform; peduncles absent or very short; bracts 2-3 mm long. Flowers: pedicels 2–6 mm long, 0·8 1·2 mm thick; calyx 4 5 mm high, 5 6 mm diam.; petals obovate to spathulate-obovate, 5 6 mm long, 2·8 5 mm wide; disk glands 0·3–1 mm long, 0·5–1 mm diam.; stamens 5 − 6 + 6 − 10; outer filaments 1 4 mm long; inner united column 4–7 mm long. Female inflorescences terminal and axillary, also terminating short lateral fertile shoots bearing immature leaves, solitary or 2-3-flowered umbels or up to 4 cm long racemes; peduncles 1 3 cm long; bracts linear-triangular, 2–7 cm long. Flowers: sepals 4 5, elliptic, oblong to obovate or narrowly ovate to lanceolate; petals 4–5, broadly oblong to suborbicular or obovate, 7–9 mm long, 5–7 mm wide; styles 5–7 mm long, united into 1–3 mm long column, free and bifid above. Capsules 12–15 mm long, 15–18 mm diam., blackish when dry; pedicels 3–10 mm long; accrescent sepals 12–33 mm long, 4 18 mm wide; seeds subtrigonous, dorsally convex, *ca* 10 mm long, *ca* 8 mm diam. (figure 4).

Flowering and fruiting: Jan.-Dec.

Specimens examined: India: Peninsular India, *sine loc. exact.*, no date, *Dalzell* s.n., Herb. Acc. nos 412189/201 (CAL). *Karnataka*: North Kanara Dist., *sine loc. exact.*, no date, *Talbot* s.n., Herb. Acc. nos 8602, 8605 (BSI); Falls of Gairsoppa, 27 Nov. 1883, *Talbot 759* (CAL); Falls of Gairsoppa, 22 Nov. 1884, *Talbot 56* (MH); north Kanara forest, May 1919, *Bell 5963* (CAL). *Kerala*: Idukki Dist., Sabarimalai, 26 Sept. 1972, *B D Sharma 42037* (MH); Travancore, near Kaldurthi, 23 May 1984, *Bourdillon 248* (CAL, MH); Travancore, no date, *Bourdillon 86* (MH); Porappar, 31 July 1913, *Rama Rao 1357* (CAL); Quilon Dist., Achenkoil, 26 May 1979, *Mohanan*

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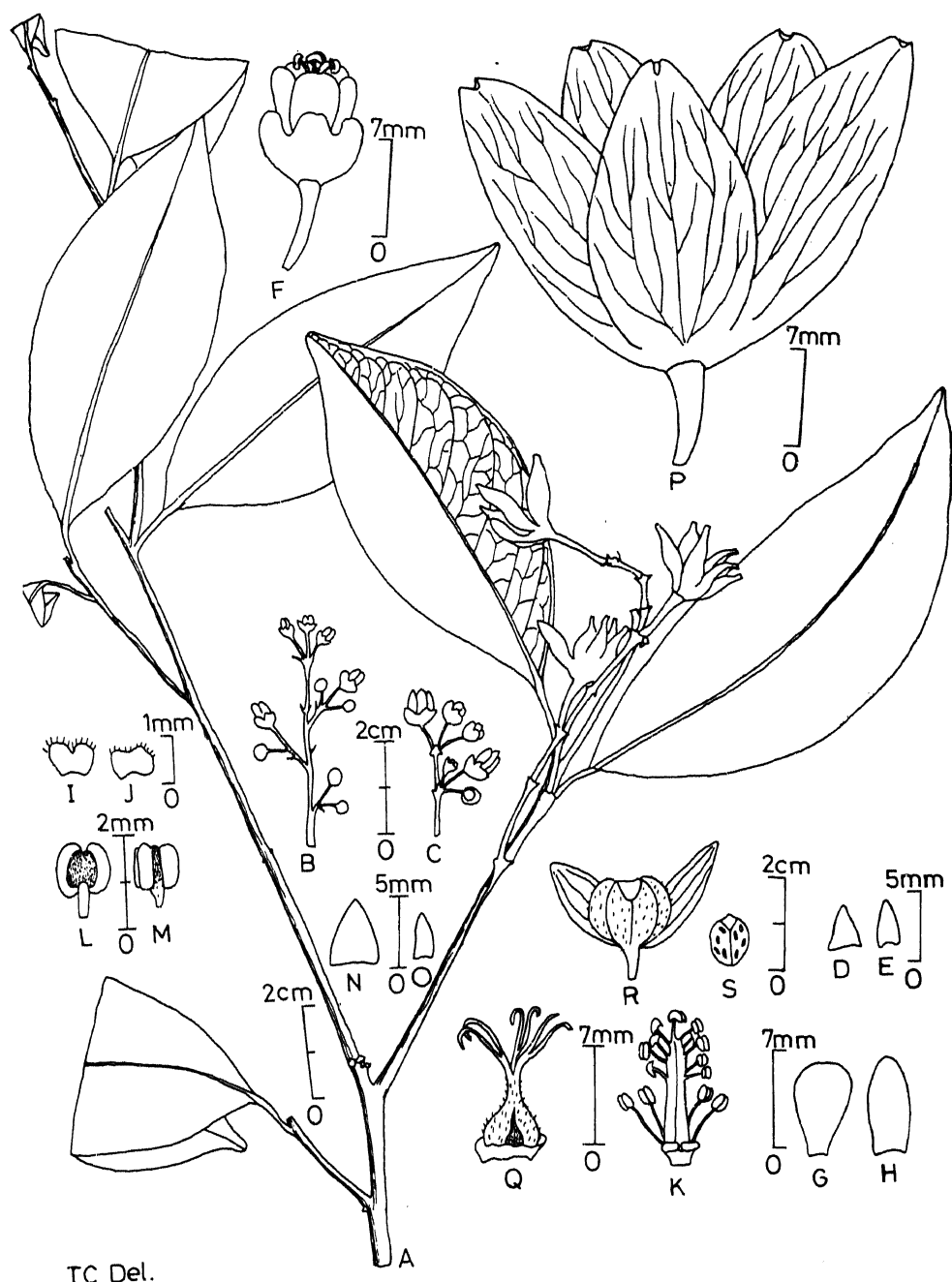
Figure 4. *D. glabellus* Thw. var. *lawianus*. A. Habit with female flowers. **B–M.** Male. **B, C.** Inflorescences. **D, E.** Bracts. **F.** Flower. **G, H.** Petals. **I, J.** Disk glands. **K.** Androecium. **L, M.** Anthers. **N–Q.** Female. **N, O.** Bracts. **P.** Flower. **Q.** Ovary with disk. **R.** Fruit. **S.** Seed.
[A. *Chandrabose* 47251 (CAL). **B, C,** D–M. *Range Officer 34* (DD). N–Q. *Talbot* s.n. (BSI). **R, S.** *Koccher* 158326 (BSI)].

63042 (CAL, MH). *Maharashtra*: Koyna, on way to Jungli Gaigad, 11 Feb. 1979, *Koccher* 158326 (BSI); Pune Dist., Kate-pani forest, Ambavone (Lanavla), 25 Mar. 1964, *Reddy* 96029 (CAL). *Tamil Nadu*: Coimbatore Dist., Karianshola, 1937, *Bor* 7909 (DD); *ibid.*, 18 Apr. 1939, *Range officer* 34, Herb. Reg. No. 82789 (DD); Ramanathapuram Dist., Nagariar estate to Sathaankoil, 13 June 1979, *Srinivasan* 63582 (CAL, MH).

Distribution: South India-Endemic.

Ecology: Scarce in evergreen forests at 150–1100 m. In some localities common as indicated in field notes.

The epithet *'lawianus'* relates back to *Croton lawianus* Nimmo (1839) a species validly published and representing a true *Croton*. The accrescent female sepals of the species probably prompted Mueller Argoviensis (1865–6) to consider it as a *Dimorphocalyx* which was recognised by him as a section of the genus *Trigonostemon*. In addition to *Croton lawianus*, Mueller also included another 3 different elements under *Trigonostemon lawianus*, in his citation of synonymy as well as some specimens. These elements segregated by Hooker (1887) are: (i) the plant under discussion, i.e. *D. lawianus*, (ii) *D. glabellus* and (iii) a species of uncertain status represented by *Falconer* 1255 (not seen). As long as we cannot pinpoint any one of these discrete elements as representing the description of Mueller and name it as lectctype, his binomial *Trigonostemon lawianus* is to be considered as *nomen confusum*. Hooker (1887) correctly segregated these elements and described them as *Croton lawianus*, *Dimorphocalyx glabellus* and *D. lawianus*. He also mentioned that the status of the material *Falconer* 1255 cited by Mueller is not known to him. Our studies indicate that all the specimens cited by Hooker under *D. lawianus* match with his description and truly belong to a single species and therefore *D. lawianus* Hook. f. is to be treated as a new species validly published by him in 1887 and the specimens cited by him are to be considered as syntypes. Hence the citation should not contain 'Muell.-Arg'. as the original author, as cited by Pax and Hoffmann (1924) The specific epithet *'lawianus'* is not preoccupied under *Dimorphocalyx* and hence there is no need for a new name. Coming to the present, the species, *D. lawianus* Hook. f. does not appear to be specifically distinct from *D. glabellus* and therefore treated herein as a variety of the latter.

Imperfectly known species

Dimorphocalyx meelboldii Pax and Hoffm. in Engler, Pfanzenr. IV, 147. xviii (Euph.-Addit. vii): 190. 1924. *Type*: Burma, Moulmein, Papun, *Meebold* 16837 (*non vidi*).

. This imperfectly known species is known only from the type collection, consisting of only fruiting material, which could not be traced and examined and therefore it is not possible to comment on its status.

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Differentiation of the seed coat in *Sesbania speciosa*

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Abstract. Structure and development of seed coat in *Sesbania speciosa* were observed by light and scanning electron microscopy. Only the outer integument forms the seed coat. The inner integument disintegrates during seed development. The epidermis of outer integument differentiates into the macrosclereid layer and the hypodermis into the osteosclereid layer. The tracheid-bar is present below the well defined and complex hilar region. The seed coat surface shows deposition of cutin in the form of rope-like upwellings which make it impermeable to water. The macrosclereid cells are packed very tightly and this compactness may also be responsible for the impermeability.

Keywords. Outer integument; inner integument; macrosclereids; osteosclereids; hilum; tracheid-bar.

1. Introduction

Sesbania speciosa Taubert Ex Engler belongs to the family Leguminosae. The seed coat of almost all the taxa belonging to this family is hard and impose impermeability. This seed coat impermeability is a major factor in preventing germination in many legume species (Rolston 1978); and is usually thought to lie in the palisade layer; it has been construed variously as a mechanical (Corner 1951) or chemical barrier due to subrization of the outer walls (Aitken 1939; Watson, 1948); to the presence of phenolics in these cells (Werker *et al* 1979); or callose in these and the nutritive cells present below these cells (Bhalla and Slattery 1984; Bevilacqua *et al* 1987; Vijayaraghavan and Seth 1990; Seth and Vijayaraghavan 1990). Clearly the development of seed coat structure is essential in understanding its nature and possible function.

The structure of the mature seed coat is well established in a number of papilionoid legumes (Hamly 1932; Aitken 1939; Martin and Watt 1944; Watson 1948; Corner 1951). Ontogeny of the seed coat and associated structures have, however been largely neglected, although the partial ontogeny of the epidermal palisade and hypodermal osteosclereids have been studied in *Pisum sativum* using both light and electron microscopy (Harris 1983, 1984). The present paper represents the detailed study of the ontogeny of the macrosclereids and osteoselereids along with the structure of hilum and tracheid-bar. The gathered observations are discussed in relation to functional aspect of the tissues.

2. Materials and methods

The seeds, during various stages of development, were fixed in 10% aqueous acrolein, dehydrated in methoxyethanol series, infiltered and embedded in glycol methacrylate resin mixture. Polymerization was accomplished at 40°C for 24 h and then at 60°C for 48 h. The embedded material was sectioned on a spencer (AO)

rotary microtome fitted with a locally made adaptor to hold glass knives. Two μm thick sections were cut and stained for histochemical studies.

Seed coat surfaces were scanned with the cuticle present and after its removal with hexane. To investigate the coat structure the seeds were immersed in liquid nitrogen for 1 min and then cracked in half. This treatment did not apparently brings about any visible artefact in the seed coat. Seed coat surfaces were coated with the layer of standard silver in a vacuum coating unit fitted with a revolving stage. Observations were made with Philips SEM 501 B Model.

3. Results

3.1 *Light microscopy*

3.1a *Seed coat*: The mature ovule of *S. speciosa* is campylotropous and bitegmic. The outer integument is well developed and consists of 4 or 5 layers of cells, with an uniseriate epidermis (figure 1A, B). Towards the micropylar region it is many layered and forms a flap-like structure. The inner integument is two layered and lyses during the seed development. The cells of the outer epidermis, during progressive stages of embryogenesis, divide periclinally and elongate in radial direction (figure 1B, C). The cells of the subepidermal layers however show both anti- and peri-clinal divisions (figure 1B) resulting in 5 or 6 layers of cells. As the seed matures, the outer epidermis differentiates into the macrosclereid layer whereas the cells of the subepidermal layer differentiates into the dumb-bell shaped osteosclereid layer. The remaining cells are however, parenchymatous. The macrosclereid cells show intensely PAS positive thickenings on the radial walls (figure 1C, D). The osteosclereid layer is affluent with thickenings on the cell equator whereas the end walls are thin. Intercellular spaces are present between these cells (figure 1E, F).

3.1b *Hilum*: The ovule at young stages is separated from the funicle by a zone of small, dense cells. Later, regional differentiation within this subfunicular meristematic zone shows distinctive features (figure 2A-C). By the late globular proembryo stage, the hilar epidermis shows differentiation into the hilar palisade layer whereas the subepidermal layers remain parenchymatous (figure 2D). The hilar palisade layer is contiguous with the counter palisade layer of the seed (figure 2E) and is medially disrupted by the hilar-fissure (figure 4A, B). Later both the palisade layers undergo radial elongation and are identical in size. The cells of the counter-palisade layer show the deposition of secondary wall materials. Cuticle is however, absent between the counter-palisade and hilar-palisade layers. The tracheid-bar develops medially and projects into the hilar-palisade through the hilar-fissure and extends from the micropyle to the chalazal end (figure 2F). The tracheid-bar differentiates only during the early dicotyledonous embryo stage. The cells of the tracheid-bar are initially small, possess dense cytoplasm, show anticlinal division and radial elongation. The deposition of the pitted and thick secondary walls in these cells commences at later stages and finally is completed at maturity (figure 2F). The cell layers around and beneath tracheid-bar dedifferentiate into arenchyma and get filled with polyphenols. The hilar-groove is surrounded by rim-aril (figure 4).
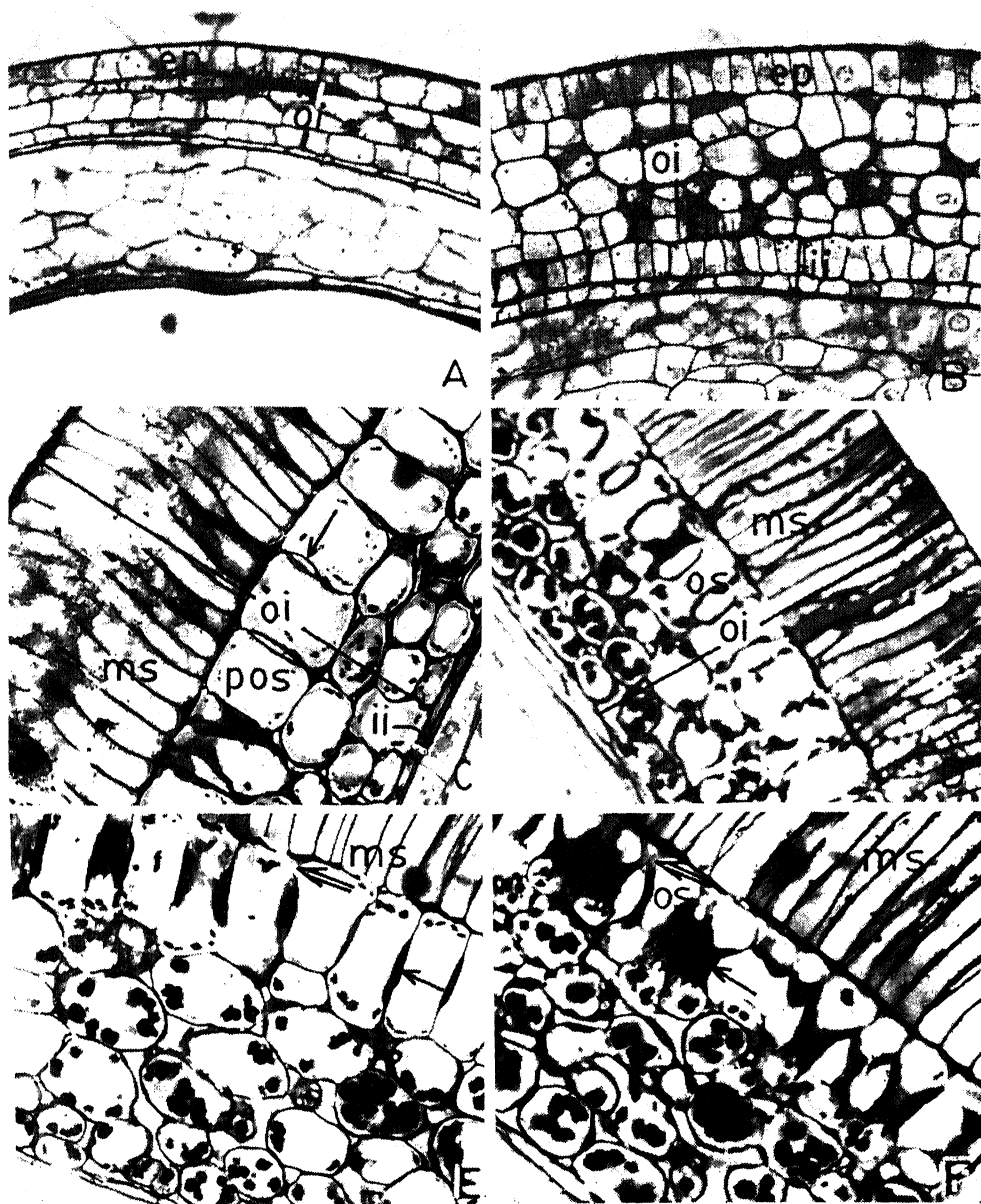
Figure 1. **A, B.** Portion of integument at mature embryo sac and preglobular proembryo stages respectively. The epidermal cells of outer integument show slight elongation. **C, D.** Osteosclereid layer at heart-shaped embryo stages to show presumptive deposition of wall materials at the cell equator (arrow in **C**). **E, F.** Osteosclereid layer at dicotyledonous embryo stage to show thin end walls (double arrow). At the cell equator a heavy deposition of wall material (arrow in **F**) occurs (× 1000).

(ep, Epidermis; ii, inner integument; oi, outer integument; os, osteosclereid; pos, presumptive osteosclereid; ms, macrosclereid).
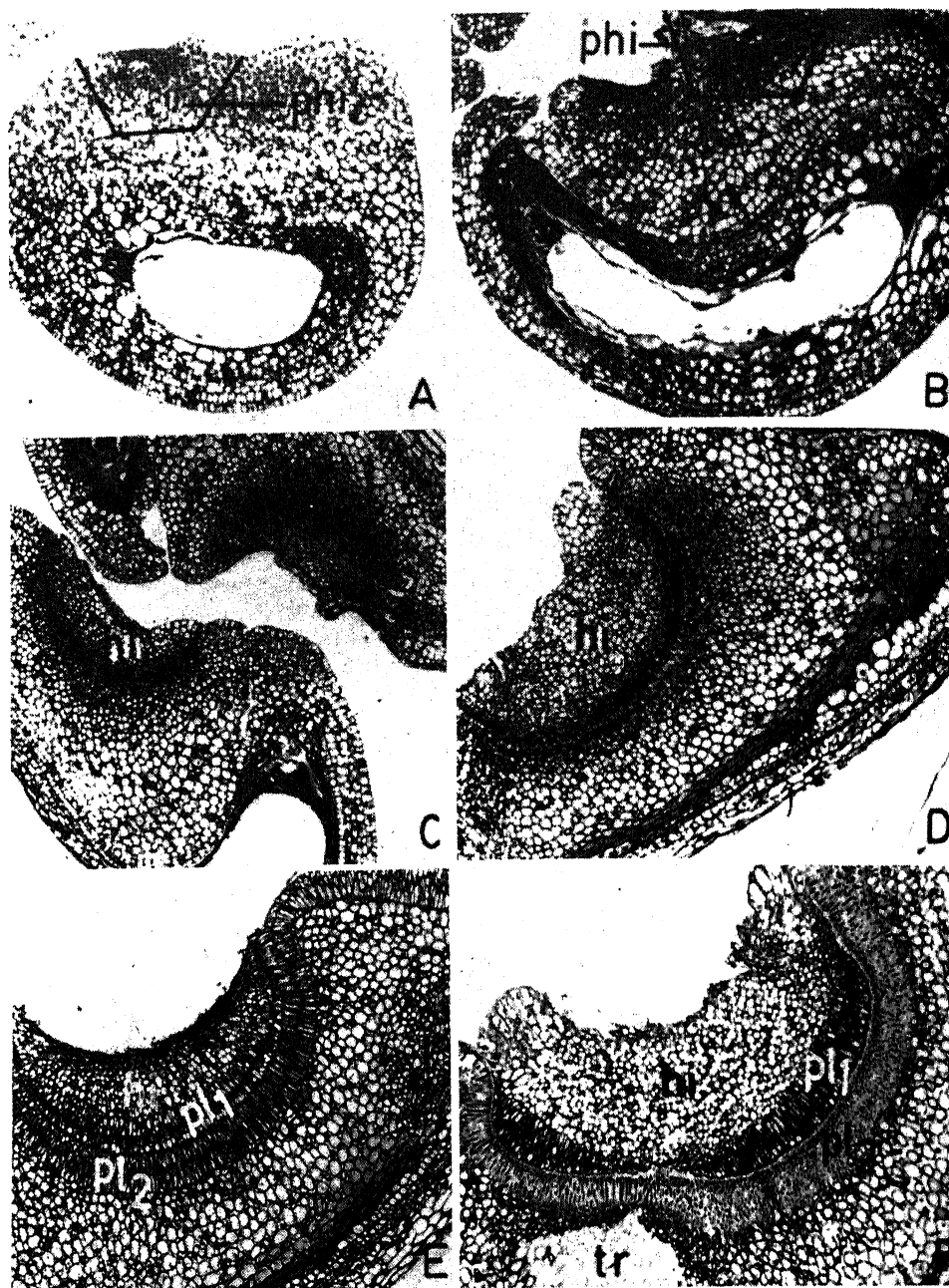
Figure 2. A-C. Longisection of ovule at mature embryo sac and developing seeds at proembryo and preglobular proembryo stages respectively to show location of hilar region (A, B × 250; C × 620). D, E. Seeds at globular proembryo and heart shaped embryo stages respectively to show the differentiation of elongate, double palisade layers. The remaining hilar cells are parenchymatous and show lax arrangement (× 620). F. The hilum is well developed at dicotyledonous embryo stage. A few cells below the palisade layer have differentiated into tracheid-bar (× 620).

(hi, Hilum; phi, presumptive hilum; pl1, palisade layer one; pl2, palisade layer two; tr, tracheid-bar).

3.2 *Scanning electron microscopy*

The surface layer consists of thin cuticle with inter-twining dendroid upwellings (figure 3A). The seeds treated with hexane lack cuticle and reveal the compact arrangement of macrosclereids (figure 3B). In the region of hilum the cuticle assumes a basket-weave like appearance (figure 4B). Beneath the cuticle lie, the cells of macrosclereid layer which under scanning electron microscope, show very solid, wall-like appearance with tight association among themselves. The osteosclereid cells are typically bone-shaped with heavy deposition of thick wall material in the region of the osteosclereid cell equator while the ends of the cells are thin walled (figure 3C). The ends of the cells, continue to expand while the equator region of the cell ceases expansion resulting in bone-shaped configuration.

4. Discussion

The histogenic sequence of the testa and the relative timing of regional differentiation observed in *S. speciosa* are common to the few papilionoid legumes studied (Coetzee and Robertse 1980; Graaff and van Staden 1983a; Harris 1983, 1984, 1987; Jain and Vijayaraghavan 1985; Manning and van Staden 1985). The epidermal cells, during differentiation into macrosclereid layer elongate and show initially PAS positive thickenings in the inner tangential and radial walls. The osteosclereids are formed by heavy deposition of thick wall material in equatorial region. The ends of the cells are, however, thin walled. In the region where the walls remain thin, continued expansion produces the typical hour-glass shape. The development of the macrosclereid and osteosclereids in *S. speciosa* is similar to that observed in *P. sativum* (Harris 1983, 1984). The radial walls of the osteosclereids get separated due to 'differential stress of growth', brought about by the circumferential growth of the macrosclereid layer. The presence of intercellular spaces in these cells is either due to the lysis of a few osteosclereid cell, or due to the expansion of parenchyma cells present below the osteosclereid layer. The adaptive value of this layer of cells with intercellular space in *S. speciosa* and other legume seed coats may be related to the weight of the seed or uptake of water and minerals during seed germination. Corner (1951) even suggested that the hour-glass layer functions in the aeration of the seed.

Differentiation of the tracheid-bar and osteosclereids coincides with final secondary wall deposition in the hilar palisade. The tracheid-bar appears to function as a central conduit to which moisture, as water vapour is channeled from throughout the seed (Manning and van Staden 1985). Hyde (1954) presented evidence that the hilar-fissure acts as a hygroscopic valve, permitting water loss from, but preventing water entry into, the maturing dehydrating seed. It has been suggested that the counter-palisade regulates opening and closing of the hilar-fissure. The counter palisade, when the relative humidity falls, dries and shrinks leaving the margins of the hilar-fissure apart. At the rise of relative humidity this layer swells and causes the closure of the fissure.

In *S. speciosa* the presence of cuticle outside the testa and the light packing of macrosclereids prevent penetration of water into the seeds. Impermeability of seeds to water has been attributed to different structures such as compactly arranged macrosclereids, thick cuticle and to substances such as tannins, waxes, pectin, lignin, hemicellulose, suberin, cutin, callose and phenolics (Marbach and Mayer 1974;
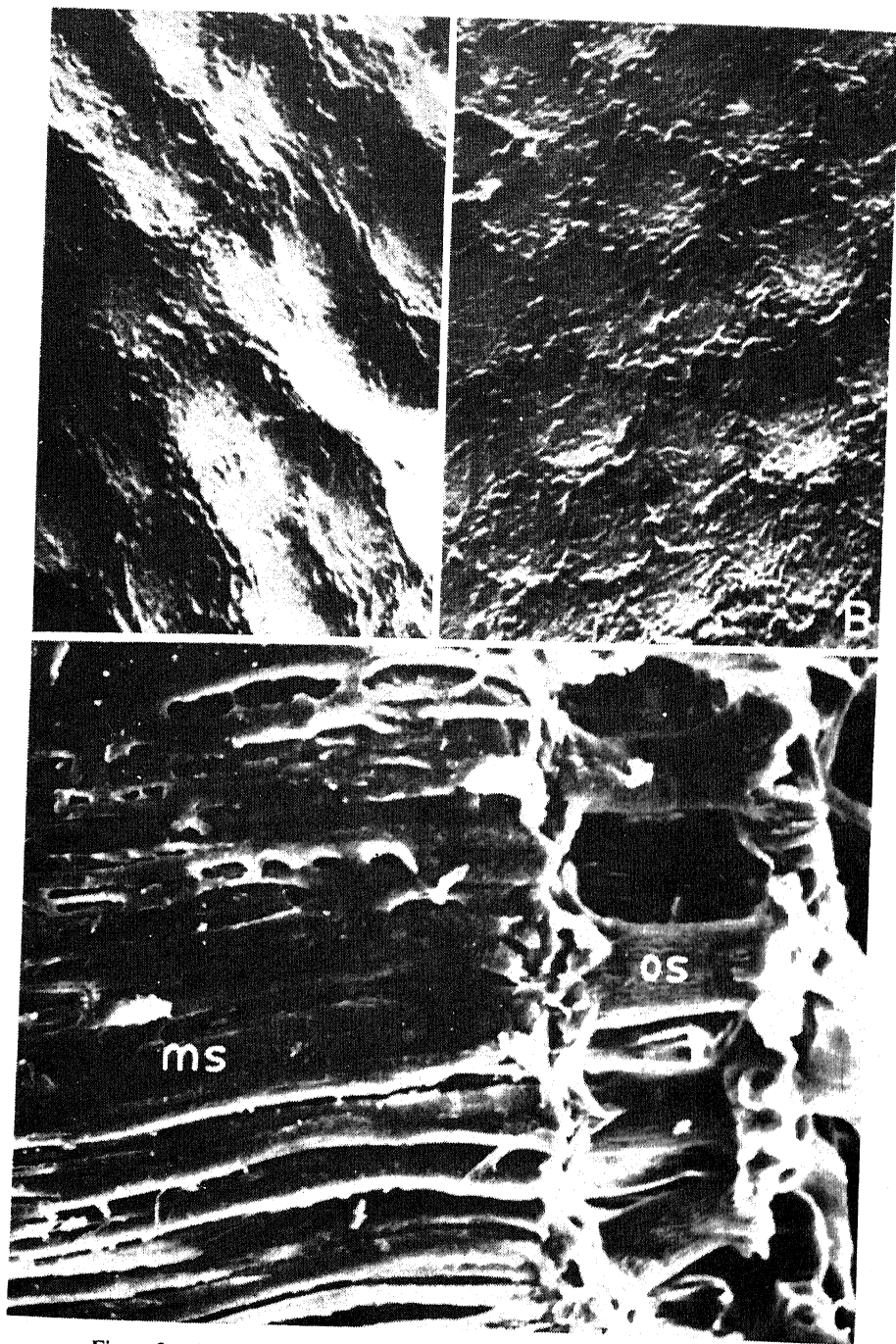
Figure 3. **A.** Testa of a dry seed showing closely packed hexagonal macrosclereid cells. Cutin appears as thread like upwellings (× 2500). **B.** Testa after treatment with hexane. The hexagonal arrangement of the macrosclereid cells, becomes evident as hexane dissolves cutin (× 2500). **C.** Fractured testa of a mature seed to show the compactly arranged macrosclereids and dumb-bell shaped osteosclereids. The secondary wall thickenings in both macro, and osteosclereids are prominent (× 1280). (ms, Macrosclereid; os, osteosclereid).
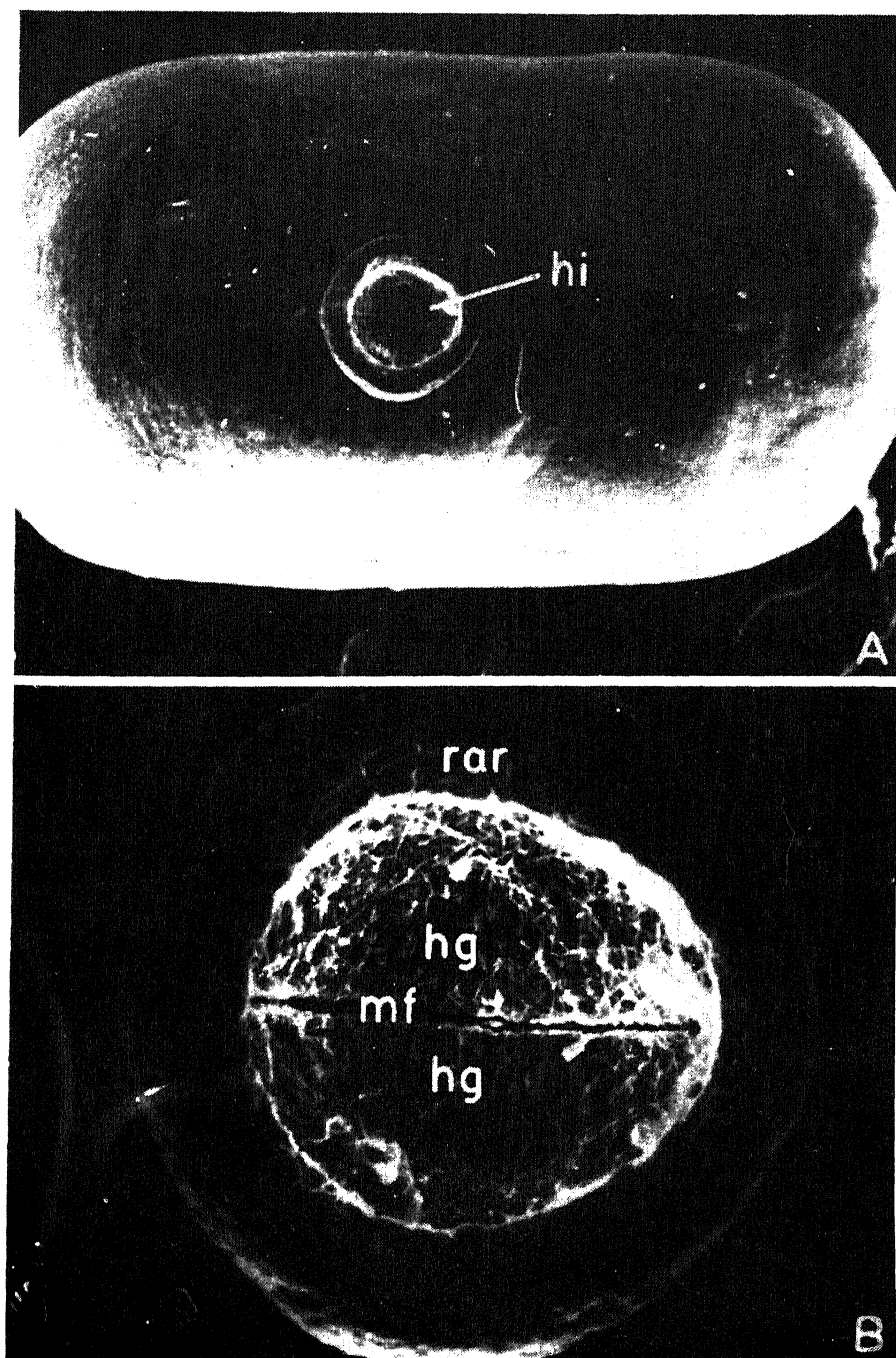
Figure 4. A. A seed showing prominent hilum. **B.** External view of the hilum showing the rim-aril and hilar groove that has a median-fissure. The hilar-groove is filled with cutin which has a basket weave like appearance (× 300).

(hg, Hilar-groove; hi, hilum; mf, median-fissure; rar, rim-aril).

Slattery *et al* 1982; Gulz and Hangst 1983; Seth and Vijayaraghavan 1990). Hard seeds, therefore require some sort of pregermination treatments such as heat shock; acid or mechanical scarification. Such treatments, which cause the permeability, do so by structurally altering the testa (Lui *et al* 1981; Graaff and van Staden 1983b) that results in increased germination.

Acknowledgements

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Boron nutrition of cowpea

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Abstract. Cowpea (*Vigna sinensis* L.) cv. Rituraj was grown in refined sand at graded levels of boron ranging from 0·0033 to 6·6 mg l^{-1}. Maximum biomass and seed weight were obtained at 0·33 mg B l^{-1} supply. In acute deficiency of boron (0·0033 and 0·033 mg l^{-1}), its deficiency symptoms were pronounced, the biomass was depressed markedly and neither pods nor seeds were formed. At moderate deficiency of boron, deficiency symptoms were mild and the number and weight of seeds were reduced by about 40% and 50% respectively. Marked boron toxicity symptoms were observed at 6·6 mg B l^{-1} supply where not only biomass and seed yield but also the concentration of starch and non-reducing sugars and the activity of starch phosphorylase and ribonuclease were depressed. In the latter treatment reducing sugars were increased so also the activity of polyphenol oxidase and peroxidase. In acute boron deficiency, the concentration of reducing sugars and non-reducing sugars were increased so also the activity of peroxidase, ribonuclease and polyphenol oxidase but the activity of starch phosphorylase was depressed only at 0·0033 mg B l^{-1} supply. As the concentration of boron in seeds was markedly less than in leaves, the values of deficiency, threshold of deficiency and threshold of toxicity in leaves was higher than in seeds.

1. Introduction

Boron deficiency is also known to adversely affect the formation and functioning of floral and fruiting organs and reduce the economic yield drastically (Hewitt 1983). Deficiency of boron not only disturbs the carbohydrate and nucleic acid metabolisms but also influences the functioning of pentose phosphate shunt (Lee and Aronoff 1967), and phenol metabolism (Shkolnik 1974).

In India, many areas are known to be deficient or marginally deficient in boron by soil tests (Kanwar and Randhawa 1974; Adriano 1986). However not all crops respond to the application of boron because its requirement for various crops varies widely (Gupta 1979; Needham 1983). Therefore it is necessary that for major crop plants, tissue boron which is indicative of its deficiency, threshold of deficiency and toxicity should be worked out as also the biochemical and physiological parameters which would indicate its deficiency or toxicity (Bouma 1983).

This paper reports the influence of variable boron on biomass, economic yield, carbohydrate fractions, some enzymes and tissue boron in leaves and seeds in cowpea (*Vigna sinensis* L.) cv. Rituraj grown in refined sand.

2. Materials and methods

Cowpea (*V. sinensis*) was grown in refined sand (Agarwala and Sharma 1976) at graded levels of boron ranging from deficiency to excess. The composition of the nutrient solution excluding boron has been already described by Agarwala *et al*

(1987). Boron as H_3BO_3 was supplied at 0·0033, 0·033, 0·165, 0·33, 3·3, 6·6 mg l^{-1}. Contribution of boron from pots, refined sand, purified nutrients and distilled water was less than 0·0033 mg l^{-1}. There were 4 replicates in each treatment. Initially two plants were maintained in each pot which were reduced to one on 34 days after sowing (DAS).

The plants were sampled at 34 DAS for biomass and tissue boron and at 84 DAS for biomass and seed yield when the concentration of tissue boron in seeds was also determined. Sugars and starch were estimated in leaves at 35 DAS; assay of activities of peroxidase, ribonuclease, starch phosphorylase and polyphenol oxidase were made in young leaves at 33 DAS along with protein in enzyme extracts as per procedure described by Chatterjee *et al* (1987). The values of deficiency, threshold of deficiency and toxicity were determined according to the procedure of Agarwala and Sharma (1979).

The data presented in the tables and figures are the means of 3 observations. Entire data was subjected to statistical analysis and tested for significance at $P = 0.05$.

3. Results

3.1 *Visible effects*

At 20 DAS, in cowpea the visible boron deficiency symptoms initiated at 0·0033 mg B l^{-1} supply as depression in growth (figure 1A). At 25 DAS young leaves of these plants developed interveinal chlorosis from the tip and margins of the lamina; later affected leaves appeared black and turned necrotic. At 35 DAS in these plants condensation of internodes, death of apical growing point, inward curling of lamina of young leaves were observed (figure 1B). The symptoms of boron deficiency were observed at 0·033 mg B l^{-1} supply 10–15 days later than at 0·0033 mg B l^{-1} supply and were less intense than in the latter treatment. In addition, at this level of boron supply yellowing of young mature leaves was also observed. At 40 DAS, chlorosis of young leaves was observed at 0·165 mg B l^{-1} supply. As far as it could be made out visually, no flowers seemed to have formed at 0·0033 and 0·033 mg B l^{-1} supply.

Cowpea raised at 3·3 and 6·6 mg B l^{-1} supply developed boron toxicity symptoms at 35 DAS, as depression in height and chlorosis of older leaves from their tips, and the intensity of these was more severe at 6·6 mg B l^{-1} supply (figure 1C).

3.2 *Dry matter*

At 34 and 84 DAS, dry matter increased significantly with an increase in boron supply from 0·0033 to 0·33 mg B l^{-1} supply. However at 84 DAS with further increase in boron supply from 0·33 to 6·6 mg l^{-1}, dry matter was decreased significantly.

No pods were formed at 0·0033 and 0·033 mg B l^{-1} supply. As compared to pod and seed yield obtained at 0·33 mg B l^{-1} supply, where both were at maximum, these were decreased significantly at 0·165 mg B l^{-1}. Number of pods and seeds,

Figure 1. **A.** Cowpea at variable boron (from left to right) at 0·0033 (deficient), 0·33 (adequate) and 6·6 (excess) mg l⁻¹. **B.** A boron deficient plant showing condensation of internodes, curling and distortion of young trifoliates and death of the apical growing point. **C.** Cowpea at excess B showing chlorosis of old leaves.

weight of total seeds and weight of 100 seeds were decreased in boron deficiency cases. The pod and seed yield were decreased slightly at excess (3.3 and 6.6 mg l^{-1}) boron supply (table 1).

3.3   *Tissue boron*

At 84 DAS boron concentration in leaves was increased with an increase in boron supply from 0.0033 to 6.6 mg l^{-1}. The concentration of boron in seeds was less than that in leaves. Seed boron also increased with an increase in boron supply from 0.165 to 6.6 mg l^{-1} (figure 3).

The values of deficiency, threshold of deficiency and toxicity in young leaves and seeds of cowpea were 9.2, 11.6 and 73 μg g^{-1} dry matter, and 6.8, 8.2 and 18 μg g^{-1} seed weight respectively (figure 2).

3.4   *Carbohydrate fractions*

In cowpea leaves, the concentration of reducing sugars at 0.0033 mg B l^{-1} was slightly less than at 0.033 B l^{-1} supply, but this difference was not significant. It was decreased markedly with an increase in boron supply from 0.033 to 3.3 mg l^{-1} where it was minimum. At 6.6 mg B l^{-1} the concentration of reducing sugars was increased significantly. The concentration of non-reducing sugars was decreased with an increase in boron supply from 0.0033 to 6.6 mg l^{-1} (table 2).

Starch concentration in leaves was significantly increased with an increase in boron supply from 0.0033 to 0.33 mg l^{-1}, where it was maximum; on increasing the

Table 1. Yield, seed properties and boron concentration in cowpea grown at variable boron supply.

| Days after sowing | mg B l^{-1} | | | | | | LSD at $P = 0.05$ |
	0.0033	0.033	0.165	0.33	3.3	6.6	
Dry matter: g plant^{-1}							
34	0.97	3.04	3.48	4.99	4.40	3.50	1.88
84	2.62	6.10	20.70	24.61	18.78	16.00	2.19
Number of pods plant^{-1}							
	—	—	8	13	13	11	—
Number of seeds plant^{-1}							
	—	—	42	89	85	71	—
g seeds plant^{-1}							
	—	—	3.88	6.76	6.23	6.18	1.40
100 seed weight (g)							
	—	—	6.24	7.59	7.34	7.29	0.59
Seed B: μg g^{-1} dry matter							
	—	—	6.5	9.0	11.5	18.0	3.8
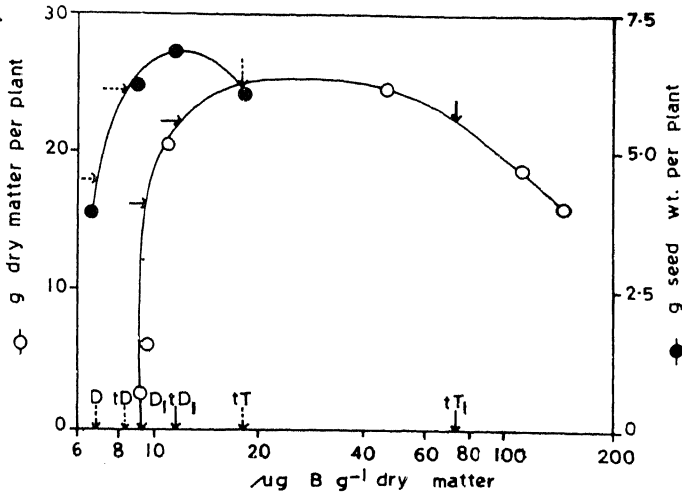
Figure 2. Values of deficiency (D), threshold of deficiency (tD) and threshold of toxicity (tT) in young leaves (○) and seeds (●) of cowpea.
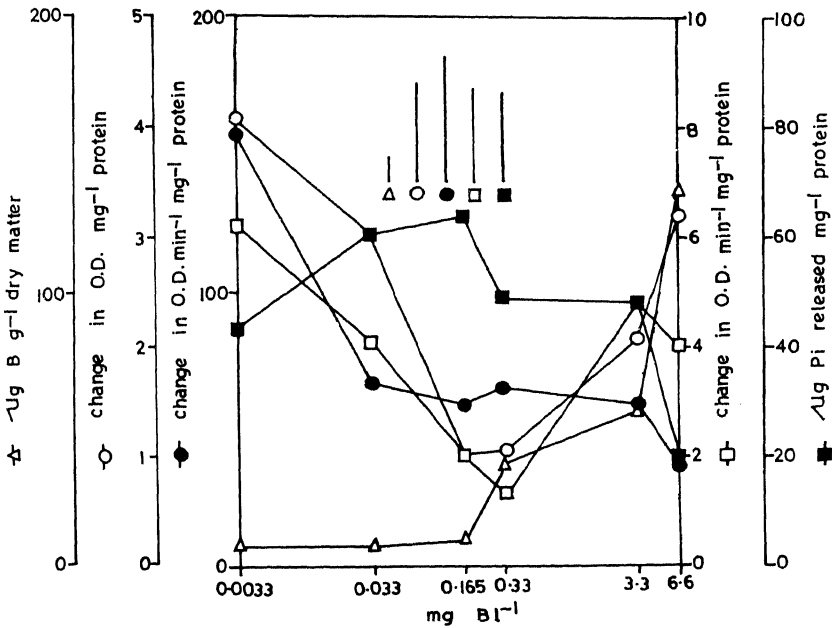
Figure 3. Boron concentration in leaves (△) in relation to the specific activity of peroxidase (○), ribonuclease (●), polyphenol oxidase (□) and starch phosphorylase (■) at variable levels of boron in cowpea. Vertical lines represent LSD $(P = 0.05)$.

boron supply from 0·33 to 3·3 mg l^{-1} its concentration was decreased significantly and further increase in boron supply to 6·6 mg l^{-1} its concentration was increased marginally (table 2).

Table 2. Concentration of different carbohydrate fractions in leaves of cowpea grown at variable boron supply.

Days after sowing	mg B l^{-1}						LSD at $P=0.05$
	0.0033	0.033	0.165	0.33	3.3	6.6	
	Per cent reducing sugars in fresh wt.						
35	0.392	0.422	0.162	0.076	0.045	0.150	0.099
	Per cent non-reducing sugars in fresh wt.						
	0.620	0.236	0.206	0.216	0.135	0.034	0.120
	Per cent starch in fresh wt.						
	1.98	2.44	2.59	4.28	1.35	1.50	1.07

3.5 *Enzyme activities*

The specific activity of peroxidase and polyphenol oxidase was significantly increased in acute boron deficiency (<0.165 mg B l^{-1}) and at excess (3.3 and 6.6 mg l^{-1}) boron supply. Whereas the activity of ribonuclease was significantly increased only at 0.0033 mg B l^{-1} supply, remained almost stationary between 0.033 and 3.3 mg B l^{-1} supply but was slightly decreased at 6.6 mg l^{-1} supply. The activity of starch phosphorylase was almost significantly increased at 0.033 and 0.165 mg B l^{-1} supply and decreased significantly at 6.6 mg B l^{-1} supply (figure 3).

4. Discussion

The visible effects of boron deficiency in cowpea were largely similar to those described for legumes other than cowpea (Sommer and Lipman 1976; Hewitt 1983). As has been observed for many plant species the decrease in biomass of cowpea in boron deficiency situation might be due to low protein formation (Mengel and Kirkby 1982). In cowpea low boron supply resulted in poor development of pods and seeds which might be accounted for by the malformed and aborted formation of embryo sacs and decreased viability of pollen grains (Agarwala *et al* 1981). With an increase in the supply of boron its concentration in leaves of cowpea continued to increase but the maximum dry weight was obtained at 0.33 mg l^{-1} boron supply when its concentration in young leaves was 37 μg g^{-1} dry matter. This is in conformity with other reports (Brennan and Shive 1948; Szabo 1979). There are several reports (Milosavljevic and Popovic 1970) that both reducing and non-reducing sugars accumulate in boron deficiency as has been observed here in cowpea but the decrease in starch concentration of leaves under boron deficiency is not in agreement with reports on pepper (Pandey *et al* 1981). The increased sugar content in leaves of boron deficient cowpea might be ascribed to impeded translocation of sugars from leaves owing to callose formation in sieve tubes and/or due to low sink activity of shoots suffering from boron deficiency (Marschner 1986) and/or due to reduced formation of sugar-borate complex in boron deficiency and/or due to stimulatory role of boron in sugar translocation (Dugger 1983). The increased activity of ribonuclease in acute boron deficiency in cowpea observed here

is in accord with the observations of Dave and Kannan (1980) for beans and this might explain lowering of RNA concentration under boron deficiency (Kevresan *et al* 1977; Dugger 1983). In boron deficient cowpea leaves there was a marked increase in the activity of polyphenol oxidase and peroxidase due to accumulation of phenols and increase in lignin precursors (Siegel 1953; Lewis 1980). Increased activity of polyphenol oxidase would inhibit auxin oxidase activity and the resulting increased amount of auxin would cause many morphological changes as noted in young growth of boron deficient cowpea.

It is not possible to explain the decreased concentration of starch at low boron supply (0·0033 to 0·165 mg B l^{-1}) on the basis of starch phosphorylase activity as its activity at lowest boron level was almost equal to that at adequate boron supply.

In acute boron deficiency visible foilar symptoms were observed and no pods or seeds were formed. Under these conditions dry weight per plant and tissue boron were decreased considerably; sugar content and activities of polyphenol oxidase and peroxidase were increased. Highest economic yield was obtained at 0·33 mg B l^{-1} supply and it was decreased by 43% at moderate deficiency of boron. At 6·6 mg B l^{-1} supply visible boron toxicity symptoms appeared and the biomass was decreased by 35% but the economic yield was depressed by less than 10%.

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Cytological explorations of Indian woody legumes

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Abstract. Cytological exploration of 47 woody leguminous species have been made from the forests of northern, central and southern India. Of these, *Acacia canescens* (n = 13), *Dichrostachys cinerea* (n = 26), *Erythrina caffra* (n = 21), *Millettia brandisiana* (n = 11), *Mucuna hirsuta* (n = 11), *Pahudia martabanica* (n = 12) and *Phanera glauca* (n = 14) are explored for the first time. Additional and/or variable cytotypes are recorded in *Bauhinia acuminata* (n = 13) and *Prosopis glandulosa* (n = 28). Existence of B-chromosomes has been recorded in *Erythrina caffra* (n = 21 + 0 − 3B), *Millettia brandisiana* (n = 11 + 0 − 2B), *Pongamia pinnata* (n = 11 + 0 − 7B) and *Tamarindus indica* (n = 13 + 0 − 4B). Besides these cytomorphological variabilities, structural heterozygosity for translocations/inversions (*Ougeinia oojeinensis*, *Pongamia pinnata*, *Saraca indica*) and cytomixis (*Caragana brevispina*) are also existent.

Keywords. Legumes; heterozygosity; translocations; inversions; cytomixis.

1. Introduction

Leguminosae, one of the largest and cosmopolitan families, has a special status in Indian forestry. Its members are well represented in various forest types. As many as 37 species provide commercial timber (Pearson and Brown 1932). *Leucaena leucocephala* (Lam.) de Wit., an exotic species with multipurpose use is quite important in social forestry in India. Besides, several species yield minor forest products and raw materials for forest based industries.

Exploration of germplasm of forest species of such a family is, therefore, desirable. The proper analysis of morphological and/or cytological-variation could provide a base for future tree improvement programmes. With this object, the present studies are undertaken from the forests of northern, central and southern India. Some exotics and cultivated members are also included.

2. Materials and methods

Chromosomal explorations have been made through meiotic studies for which flower buds were fixed in Carnoy's fluid. Smears were prepared using standard acetocarmine techniques. Pollen fertility was estimated on the basis of their well-filled nature and stainability with glycero-acetocarmine (1:1) mixture.

3. Results and discussion

Information on specific locality, chromosome number, ploidy level, pollen fertility and previous reports of the presently investigated 47 species is provided in table 1. Voucher specimens are available in the Herbarium, Department of Botany, Punjabi

Table 1. Chromosomal data of the presently investigated species.

Taxa	Locality	Voucher specimen number	Chromosome number	Previous reports*
Acacia Willd. (x = 13)				
A. caesia W. and A.	Pachmarhi: Duchess Fall, 800 m[a]	23191	n = 13	2n = 26: Bir and Kumari (1978)
A. canescens Grah.	Kodaikanal: Fall's view, 800 m[a]	29292	n = 13	
A. dealbata Link.	Kodaikanal: Piller Rocks, 800 m[a]	29296	n = 13	2n = 26: Ghimpu (1929c,d,1930); Atchison (1948)
A. decurrens Willd.	Kodaikanal: Piller Rocks, 800 m[a]	28355	n = 13	2n = 26: Ghimpu (1929c,d,1930); Atchison (1948); Brigs vide Fedorov (1969)
Albizia durazz. (x = 13)				
A. lebbeck Benth.	Kodaikanal: Kodai Road, 250 m[a]	29177	n = 13	2n = 26: Patil (1958); Mehra and Hans (1971, 1972); Mehra (1972); Mehra and Sareen (1973)
Bauhinia Linn. (x = 13, 14)				
B. acuminata Linn.	Dehra Dun: F.R.I., 600 m (cult.)[a]	23001	n = 13	2n = 28: Pantulu (1942); Atchison (1951); Sharma and Raju (1968); Bir and Kumari (1979)
B. galpini N. E. Brown	Dehra Dun: F.R.I., 600 m (cult.)[a]	23502	n = 14	2n = 28: Atchison (1948); Rao (1954); Sharma and Raju (1968)
B. retusa Buch.-Ham.	Mussoorie: Jharipani, 1,500 m[a]	23281	n = 14	2n = 24, 26, 28: Sharma and Raju (1968); 2n = 28: Pantulu (1942); Atchison (1951); Rao (1967); Mehra and Sareen (1973); Sandhu and Mann (1988).
Butea Roxb. ex Willd. (x = 9)				
B. monosperma (Lam.) Taub. (= *B. frondosa* Roxb.)	Pachmarhi: Matkuli, 450 m[a]	25947	n = 9	2n = 18: Rao (1954); Raghavan and Arora (1958); Nanda (1962); Tixier (1965); Bir and Sidhu (1967); Mitra and Datta (1967); Mehra and Sareen (1973); Sanjappa and Bhatt (1976); Sinha and Kumar (1978); Anis (1983); Sandhu and Mann (1988); 2n = 18 + 1f: Kedharnath (1950); 2n = 18 + 1B: Anis (1983)
	Patiala Theri, 250 m[a]	25946	n = 9	
Caesalpinia Linn. (x = 11, 12)				
C. decapetala (Roth.) Alston	Pachmarhi: Jambu Dwip, 1,000 m[a]	25951	n = 12	2n = 22: Malla *et al* (1977); 2n = 24: Rao (1967); Bir and Kumari (1973, 1977);

(*table 1 contd.*)

Taxa	Locality	Voucher specimen. number	Chromosome number	Previous reports*
(= C. sepiaria Roxb.)	Dehra Dun: Lachhiwala, 600 m[a]	—	n = 12	Gill *et al* (1982); Sandhu and Mann (1988)
	Kodaikanal: Dolmen Circle, 1400 m[a]	29357	n = 12	
Campylotropis Bunge (x = 9, 11)				
C. *Stenocarpa* (Koltz.) Schind. (= *Lespedeza stenocarpa* Maxim.)	Saharangpur: Mohand, 400 m[a]	22811	n = 11	2n = 18: Mehra and Dhawan (1971); 2n = 22: Sandhu and Mann (1988)
Caragana Lam. (x = 8)				
C. *brevispina* Royle	Chamoli: Gobind Dham, 3,000 m[a]	22800	n = 8	2n = 16: Fedorov (1969); Sandhu and Mann (1988)
	Yamnotri, 3,300 m[b]	26329	n = 8	
Cassia Linn. (x = 7, 8)				
C. *auriculata* Linn.	Kodaikanal: Periyakulum, 800 m[c]	29347	n = 14	2n = 14, 16: Jacob (1940); 2n = 14, 16, 28: Irwin and Turner (1960); 2n = 28: Pantulu (1940, 1960a); Tandon and Bhat (1970)
C. *fistula* Linn.	Kodaikanal: Otthu, 1,300 m[c]	29137	n = 14	2n = 24: Tischler (1921–22); Irwin and Turner (1960); Nanda (1962):
	Dolmen Circle, 1,400 m[c]	29139	n = 14	2n = 26: Bir and Sidhu (1967); 2n = 28: Pantulu (1946, 1960a); Irwin and Turner (1960); Tandon and Bhat (1970); Mehra and Hans (1971); Bir and Kumari (1973); Datta and Datta (1973); Mehra and Sareen (1973)
C. *occidentalis* Linn.	Kodaikanal: Tiger Shola, 1,800 m[c]	29129	n = 12	2n = 26: Muto (1929); Frahm-Leliveld (1960); Irwin and Turner (1960); Miège (1962); Bir and Sidhu (1967); Gupta and Gupta (1971); Sinha *et al* (1972); Sinha and Prasad (1973)
				2n = 28: Senn (1938b); Pantulu (1940, 1960); Turner (1956); Irwin and Turner (1960); Miège (1962); Hsu (1967); Tandon and Bhat (1970); Randell (1970); Gupta and Gupta (1971); Larsen (1971); Sareen *et al* (1974)

(table 1 contd.)

Taxa	Locality	Voucher specimen number	Chromo-some number	Previous reports*
Crotalaria Linn. (x = 7, 8)				
C. verrucosa Linn.	Kodaikanal: Fall's view, 800 m[a]	—	n = 8	2n = 16: Raghavan and Venkatasubban (1943); Atchison (1950); Datta and Biswas (1963); Tandon and Bhat (1970); Subramanian (1972); Bairiganjan and Patnaik (1989)
Dalbergia Linn. f. (x = 10)				
D. lanceolaria Linn.f.	Rishikesh: Narendra Nagar, 600 m[a]	22999	n = 10	2n = 20: Atchison (1951); Mehra and Hans (1972); Sarkar *et al.* (1974);
	Kodaikanal: Fall's View, 800 m[a]	29152	n = 10	Bir and Kumari (1975); Sanjappa and Dasgupta (1977); Sinha and Kumar (1978)
D. latifolia Roxb.	Dehra Dun: F.R.I., 600 m (cult.)[d]	23271	n = 10	2n = 20: Atchison (1951); Mehra and Hans (1971, 1972); Sanjappa and Dasgupta (1981)
D. paniculata Roxb.	Pachmarhi 1,050 m[a]	26057	n = 10	2n = 20: Atchison (1951); Bir and Kumari (1977)
D. sericea G. Don. (= *D. hircina* Benth.)	Dehra Dun: Sahasradhara, 600 m[a]	22993	n = 10	2n = 20: Rao (1967); Mehra and Hans (1971, 1972); Mehra and Sareen (1973)
D. sissoo Roxb.	Dehra Dun: Ballupur, 600 m[a]	23029	n = 10	2n = 20: Patil (1958); Nanda (1962); Rao (1967); Mehra and Hans (1971, 1972); Mehra and Sareen (1973); Bir and Kumari (1977); Sandhu and Mann (1988); Bairiganjan and Patnaik (1989)
Delonix Rafin (x = 12, 14)				
D. regia (Boj.) Rafin.	Kodaikanal, 2,050 m[a]		n = 12	2n = 24: Poucques (1945a): 2n = 28: Jacob (1940); Atchison (1951); Berger *et al* (1958); Mehra and Sareen (1973)
Desmodium Desv. (x = 10, 11)				
D. elegans DC. (= *D. tiliaefolium* G. Don)	Mussoorie, 2,050 m[a]	23241	n = 11	2n = 22: Bir and Sidhu (1967); Rao (1967); Rotar and Urata (1967); Koul and Gohil (1973); Mehra and Sareen (1973); Sanjappa and Bhatt (1977); Bir and Kumari (1979); Sandhu and Mann (1988)
D. latifolium DC.	Dehradun:	23150	n = 11	2n = 22: Young (1940); Rotar

(*table 1 contd.*)

Taxa	Locality	Voucher specimen number	Chromo- some number	Previous reports*
(= *D. velutinum* (Willd.) DC.)	Lachhiwala, 600 m[a]			and Urata (1967); Mehra and Dhawan (1971); Bir and Kumari (1973, 1977); Sanjappa and Bhatt (1977)
D. rufescens DC. (x = 13)	Kodaikanal: Periyar Shola, 1,000 m[a]	29350	n = 11	2n = 22: Bir and Sidhu (1967)
D. cinerea W. and A.	Kodaikanal: Otthu, 1,300 m[c]	29447	n = 26	
Erythrina Linn. (x = 21)				
E. caffra Thunb.	Dehra Dun: F.R.I., 600 m (cult.)[d]	22832	n = 21 + 0 − 3B	2n = 42: Atchison (1947); Krukoff (1969)
E. indica Linn.	Kodaikanal, 2,100 m[a]	29281	n = 21	2n = 42: Rao (1945); Atchison (1947); Nanda (1962); 2n = 44: Poucques (1945a),
Indigofera Linn. (x = 7, 8) *I. gerardiana* R. Grah. (= *I. heterantha* Wall.)	Chakrata: Jadi, 2400 m[e]	23441 23441	n = 24 n = 24	2n = 16: Bir and Sidhu (1967); Baquar and Abid Askari (1970a,b); Bir and Kumari (1979); Sandhu and Mann (1988); 2n = 32: Kumari *et al* (1989); 2n = 48: Kreuter (1929, 1930); Fram-Leliveld (1957, 1960); Sanjappa and Bhatt (1977); Bir *et al* (1982).
I. hebepetala Benth. ex Baker	Yamnotri, 3,300 m[a]	26322	n = 8	2n = 16: Bir *et al* (1982); Sandhu and Mann (1988)
I. pulchella Roxb. (= *I. cassioides* Rottl. ex DC.)	Dehra Dun: F.R.I., 600 m[a] (cult.)	22976	n = 8	2n = 16: Patil (1958); Bir and Sidhu (1967); Mitra and Datta (1967); Rao (1967); Bir and Kumari (1977); Sareen and Trehan (1977); Sandhu and Mann (1988)
Lonchocarpus Kunth (x = 11) *L. neuroscapha* Benth.	Dehra Dun: F.R.I., 600 m[a] (cult.)	22802	n = 11	2n = 22: Atchison (1949)
Millettia W. and A. (x = 8, 10, 11, 12) *M. brandisiana* Kurz.	Dehra Dun: F.R.I., 600 m[b] (cult.)	22830	n = 11 + 0 − 2B	
M. extensa Benth. ex Baker	Dehra Dun: Mothronwala,	22997	n = 11	2n = 20: Atchison (1951); Sanjappa and Dasgupta (1977);

(table 1 contd.)

Taxa	Locality	Voucher specimen number	Chromo- some number	Previous reports*
(= *M. auriculata* Baker ex. Brandis)	600 m[a] Saharanpur: Mohand, 400 m[a]	23010	$n = 11$	$2n = 22$: Bir and Kumari (1973, 1977).
M. ovalifolia Kurz.	Dehra Dun: F.R.I., 600 m (cult.)[a]	23000	$n = 11$	$2n = 20$: Atchison (1951); Sanjappa and Dasgupta (1977) $2n = 22$: Pal (1960); Findley and McNeil (1974); Sareen *et al* (1974, 1980); Sanjappa and Dasgupta (1977); Bir and Kumari (1979)
Mimosa Linn. (x = 13)				
M. himalayana Gamble	Dehra Dun: Bindaal, 600 m[a]	23292	$n = 13$	$2n = 26$: Bir and Kumari (1978)
Mucuna Adans. (x = 11) *M. hirsuta* W. and A.	Kodaikanal: Perumalmalai, 1000 m[a]	29293	$n = 11$	
Ougeinia Benth. (x = 11, 12) *O. oojeinensis* (Roxb.) Houch. (= *O. dalbergioides* Benth.)	Pachmarhi: Matkuli, 450 m[d]	23554	$n = 11$	$2n = 22$: Sareen and Trehan (1976, 1977); $2n = 24$: Mehra and Sareen (1973); Bir and Kumari (1977)
Pahudia Miq. (x = 12) *P. martabanica* prain	Dehra Dun: F.R.I., 600 m[a] (cult.)	22828	$n = 12$	
Peltophorum Vogel (x = 13, 14) *P. africanum* Sond.	Dehra Dun: F.R.I., 600 m[f] (cult.)	23009	$n = 13$	$2n = 26$: Turner and Fearing (1959); Bir and Kumari (1979)
Phanera Lour. (x = 14) *P. glauca* Wall. ex (= *Bauhinia glauca* Wall. ex. Benth.)	Dehra Dun: F.R.I., 600 m[g] (cult.)	22812	$n = 14$	
Poinciana Linn. (x = 11, 12) *P. pulcherrima* Linn.	Kodaikanal: Palni, 400 m[a]	29171	$n = 12$	$2n = 22$: Bir and Sidhu (1967); $2n = 24$: Senn (1938); Jacob (1940); Atchison (1951); Berger *et al* (1958); Bir and Kumari (1973); Sareen *et al* (1974)

(table 1 contd.)

Taxa	Locality	Voucher specimen number	Chromo-some number	Previous reports*
Pongamia Vent. (x = 11)				
P. pinnata (Linn.) Pierre	Pachmarhi:	22538	n = 11 +	2n = 20: Atchison (1951);
(= P. glabra Vent.)	Matkuli, 450 m (cult.)*b*		0 – 7B	2n = 22: Patel and Narayana (1937); Raghavan and Arora (1958); Mehra and Hans (1971), Sanjappa and Bhatt (1976); Bir and Kumari (1977), Sarbhoy (1977); Bairiganjan and Patnaik (1989)
Prosopis Linn. (x = 13,14)				
P. glandulosa Torr. var. torreyana	Kodaikanal: Kodai road, 250 m*c*	29529	n = 28	2n = 26: Ramanathan (1950); 2n = 28: Baquar et al (1966)
Saraca Linn. (x = 12)				
S. indica Linn.	Dehra Dun: F.R.I., 600 m (cult.)*d*	26315	n = 12	2n = 24: Pantulu (1943); Atchison (1951); Simmonds (1954); Mehra and Hans (1971, 1972); Bir and Kumari (1979); Sanjappa and Dasgupta (1981)
Tamarindus Linn. (x = 12)				
T. indica Linn.	Pachmarhi: Singhanama, 500 m*h*	26313	n = 12 + 0 – 4B	2n = 24: Paul (1937); Atchison (1951); Mehra and Hans (1971); Mehra and Sareen (1973); Bir and Kumari (1977); Sanjappa (1978); Hussaini and Gill (1985)
Wisteria Nutt. (x = 8)				
W. sinensis (Sims) DC.	Dehra Dun: F.R.I., 600 m (cult.)*i*	22977	n = 8	2n = 16: Roscoe (1927); Bir and Kumari (1975)

*Darlington and Wylie (1955); Index to plant chromosome numbers (1956–1974); Löve and Löve (1961, 1974, 1975); Fedorov (1969); Chromosome number reports published in Taxon and Journal of Cytology and Genetics and Biological Abstracts.

Ploidy level and pollen fertility: [a]2x, 100%; [b]2x, 80%; [c]4x, 100%; [d]2x, 82%; [e]6x, 100%; [f]2x, 98%; [g]2x, 57%; [h]2x, 30%; [i]2x, 51%.

University, Patiala with PUN as abbreviation. Features of cytological and/or morphological interest discussed under each genus.

3.1 *Acacia* Willd.

A genus with 750–800 species is of considerable forestry importance. Of the 25

Indian species, 5 yield commercial timber. Besides, several species are introduced for afforestation. Of the 4 species studied, *A. canescens* with n = 13 is counted for the first time. Whereas in *A. dealbata*, *A. decurrens* and *A. caesia* the chromosome report of n = 13 agrees with the earlier reports (table 1).

3.2 *Albizia* Durazz.

A genus of forestry importance with 6 species provide commercial timber. *A. lebbeck* is widely distributed in India up to 1,600 m. Also, it is planted as an avenue tree. All the presently studied populations from south India are diploid with n = 13 and it agrees with the earlier reports from eastern and western Himalaya.

3.3 *Bauhinia* Linn.

A genus of 300 species of trees and shrubs is of some forestry importance. Of the 3 species explored chromosomally, the count of n = 13 for *B. acuminata* from cultivated plants establishes a new cytotype against the earlier record of n = 14 whereas for *B. galpini* and *B. retusa*, the present count of n = 14 agrees with the earlier reports.

3.4 *Butea* Roxb. ex Willd.

Of the 3 Indian species, *B. monosperma* is distributed throughout India in the plains and up to 900 m in the hills. Various forms on the basis of flower colour as red, yellow and orange, as reported presently and white flowered as reported by Kamran (1989) are existent. However, cytologically all the Indian populations inclusive of the present one are diploid with n = 9. However, B-chromosomes exist in some populations of Gwalior forests (Anis 1983).

3.5 *Caesalpinia* Linn.

C. decapetala, a large straggling thorny shrub, is widely distributed in the tropical and sub-tropical forests. All the Indian populations inclusive of those studied presently are diploid with n = 12. However, a cytotype with n = 11 also exists in Nepal (Malla *et al* 1977).

3.6 *Campylotropis* Bunge

C. stenocarpa is widely distributed in the western Himalaya. The presently studied population from Dehradun forests and those studied by Sandhu and Mann (1988) from Shimla hills are diploid with n = 11. However, a cytotype with n = 9 also exist in Nainital hills (Mehra and Dhawan 1971).

3.7 *Caragana* Lam.

Of the 10 Indian species, *C. brevispina* forms the important constituent of cold

temperate forests. Majority of the populations studied presently show the same chromosome number n = 8 and normal meiosis resulting into good pollen and seed fertility. However, some individuals in the forests of Gobind Ghat show cytomixis resulting in the variation of chromosome number (n = 4 to n = 15) and meiotic irregularities. The populations studied from Shimla hills by Sandhu and Mann (1988) are also diploid (n = 8).

3.8 *Cassia* Linn.

A genus with 500 species of trees, shrubs and herbs, is of considerable forestry importance. All the 3 species, *C. auriculata*, *C. fistula* and *C. occidentalis* explored presently are variable chromosomally (table 1). In *C. auriculata* the presently studied plants from Palni hills and from other parts of India are tetraploid with n = 14. Outside India, the species is also known to have diploid cytotypes with 2n = 14 and 2n = 16. Majority of the Indian populations of *C. fistula* inclusive of the present one are counted to have n = 14. Other chromosomal races with n = 12 and n = 13 also exist in India. However, for *C. occidentalis*, the present count of n = 12 from south India establishes a new cytotype to the already existing cytotypes with n = 13 and n = 14.

3.9 *Crotalaria* Linn.

C. verrucosa is distributed in the tropical regions. All the populations inclusive of the present one are diploid with n = 8.

3.10 *Dalbergia* Linn. f.

A large woody genus with 300 species is important in Indian forestry. Five species, *D. lanceolaria*, *D. latifolia*, *D. paniculata*, *D. sericea* and *D. sissoo* are studied presently. Of these, morphological variations based on growth and habit are recorded in *D. sissoo*. Cytologically all the species inclusive of the morphotypes of *D. sissoo* are counted to have the same chromosome number (n = 10) and regular meiosis. Other Indian populations of these species studied so far are also diploid with 2n = 20 (table 1).

3.11 *Delonix* Rafin.

D. regia, a native of Malagasy is commonly planted as an avenue tree. The present count of n = 12 from south India adds a new cytotype to the already existing cytotype with n = 14.

3.12 *Desmodium* Desv.

A genus of herbs and shrubs is of forestry importance as its members are well represented in Indian tropical, subtropical and temperate forests. The present counts of n = 11 for *D. elegans*, *D. latifolium* and *D. rufescens* agree with the earlier records from other populations (table 1).

3.13   *Dichrostachys* DC.

D. cinerea, a woody shrub is explored from the Palni hills. The species which is at tetraploid level (n = 26) shows normal meiosis and high pollen and seed fertility. It is counted chromosomally for the first time.

3.14   *Erythrina* Linn.

A genus of shrubs and trees is of some forestry importance. The present count of n = 21 for *E. indica* from south India agrees with the earlier records. However, cytotype with 2n = 44 (Poucques 1945) exists outside India. *E. caffra* an exotic species which is counted for the first time from India is also diploid with n = 21. During meiosis 21 bivalents are regularly constituted at M–I. These trees show 1–3 B-chromosomes in some PMCs. Although Bs show pairing, they lag at A–I. Pollen sterility (23%) in these trees could be attributed to these lagging Bs.

3.15   *Indigofera* Linn.

A genus of herbs and shrubs form the constituent of ground vegetation in tropical, subtropical and temperate forests. Three species are counted chromosomally. Of these *I. gerardiana* is variable cytologically with diploid (2n = 16), tetraploid (2n = 32) and hexaploid (2n = 48) cytotypes. The presently explored populations from Garhwal Himalaya are hexaploid (n = 24) with regular meiosis and 100% pollen fertility. Other two species, *I. hebepetala* and *I. pulchella* counted presently (n = 8) and by other workers do not show any chromosomal diversity.

3.16   *Lonchocarpus* Kunth

L. neuroscaphá, an exotic species, is diploid with n = 11 and is the first record from India.

3.17   *Millettia W. and A.*

The genus is represented by 180 species of shrubs and trees in tropics and subtropics. Of the 3 species studied presently, *M. brandisiana* (n = 11) is counted for the first time, whereas *M. extensa* and *M. ovalifolia* which are also diploid with n = 11, earlier records are confirmed. However in both the species, cytotype with n = 10 also exists in Indian populations. In *M. brandisiana* besides 11 bivalents, some pollen mother cells (PMCs) have two univalents which lag during A–I. B-chromosomes are also present in some PMCs. Some pollen sterility (20%) in these trees could be attributed to these lagging chromosomes.

3.18   *Mimosa* Linn.

It is a large genus with 450–500 species of diverse habit ranging from herbs, shrubs to trees. *M. himalayana*, studied from Dehra Dun and Saharanpur is counted to

have n = 13 and agrees with the earlier records from Pachmarhi hills (Bir and Kumari 1979).

3.19 *Mucuna* Adans.

M. hirsuta, a woody climber with ferrugineous hairs is counted chromosomally for the first time and is diploid with n = 11.

3.20 *Ougeinia* Benth.

A monotypic genus represented by *O. oojeinensis* provides excellent commercial timber. The presently studied populations from Pachmarhi hills unequivocally reveal 11 bivalents at M–I. Some pollen sterility (23%) in these populations is due to chromatin bridges, laggards and fragments in some PMCs. Bir and Kumari (1977) reported the cytotype with 2n = 24 from the same area. These cytotypes with 2n = 22 and 2n = 24 also exist in north India indicating that these chromosomal races have been well stabilized in India.

3.21 *Pahudia* Miq.

M. martabanica, an exotic species is counted to be diploid with n = 12. The genus is counted chromosomally for the first time.

3.22 *Peltophorum* Vogel.

The diploid count of n = 13 for *P. africanum* agrees with earlier records from India and elsewhere.

3.23 *Phanera* Lour.

P. glauca, an exotic species is counted from the cultivated individuals. The meiotic count of n = 14 is a first report for the species. High pollen sterility (43%) is attributed to some genic reasons as meiotic course is perfectly regular.

3.24 *Poinciana* Linn.

P. pulcherrima treated earlier as *Cassia pulcherrima*, is commonly cultivated. All the individuals explored so far including the present one are diploid with n = 12. However, a cytotype with 2n = 22 also exist in India (Bir and Sidhu 1967).

3.25 *Pongamia* Vent.

P. pinnata, a tree species of commercial timber importance is also planted in avenues. Explorations from north and central Indian plantations revealed that the species is variable morphologically and two forms based on flower colour exist in the Punjab plains. Though cytotype with 2n = 20 (Atchison 1951) exist elsewhere,

the Indian plants invariably have n = 11. Multiple association of chromosomes have been frequently observed earlier (Sarbhoy 1977; Bir and Kumari 1979) and in the present study. This suggests that the species is quite amenable to structural changes of chromosomes. B-chromosomes have also been observed in the present investigations.

3.26 *Prosopis* Linn.

P. glandulosa, an exotic species is introduced for afforestation purposes. The presently studied plantations fall under the var. *torreyana* and are found to be tetraploid with n = 28 having normal meiosis and high pollen fertility. This is the first record of tetraploid cytotype to the already existing diploid cytotypes with n = 13 (Ramanathan 1950) and n = 14 (Baquar *et al* 1966).

3.27 *Saraca* Linn.

S. indica a tree of eastern Himalaya as counted from cultivated individuals show n = 12 as reported by other workers. Mehra and Hans (1971) however, located some structural hybrids in Khasi hills.

3.28 *Tamarindus* Linn.

A monotypic genus represented by *T. indica*, is indigenous to tropical Africa. However it has naturalized in all parts of India. All the Indian populations inclusive of the present one are diploid with n = 12. However, some individuals in Pachmarhi have 1–4 B-chromosomes. These individuals show pollen malformation up to 30%.

3.29 *Wisteria* Nutt.

W. sinensis, an exotic ornamental species is counted to have n = 8 as reported earlier. High pollen sterility (49%) in spite of regular meiotic course could be attributed to various genic or ecological factors as is the case in several exotic species.

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Sorghum nitidum (Vahl) Pers., occurrence, morphology and cytology*

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Abstract. The distribution, collection, morphology and cytology of *Sorghum nitidum* (Vahl) Pers. belonging to the section *Parasorghum* of genus *Sorghum* are presented. The species collected in the western ghats of Tamil Nadu and Kerala has 10 normal (A) chromosomes (2n = 10) and 3 supernumerary (B) chromosomes, which are reported for the first time in Indian collections.

Keywords. *Parasorghum*; *Sorghum nitidum*; B chromosomes.

1. Introduction

Sorghum Moench is an immensely variable genus, and was sub-divided into sections *Chaetosorghum*, *Heterosorghum*, *Parasorghum*, *Sorghum* and *Stiposorghum* (Garber 1950). The species belonging to the section *Parasorghum* are the least known among the genus *Sorghum* and they have been studied very little so far (Rangaswami Ayyangar and Ponnaiya 1941). The Parasorghums are distinguished from the Sorghums (true sorghums) by bearded sheath nodes and a reduced chromosome number 2n = 10 as against 2n = 20 in the latter. Little work has been done on the cytology of these wild sorghum types, probably because of the non-availability of viable seed in any germplasm bank in the world. One such species, *Sorghum nitidum*, belonging to the section *Parasorghum* is reported to be distributed in the southern portion of Asia and tropical Australia (Rangaswami Ayyangar and Ponnaiya 1941). Two accessions of this species were collected in the western ghats in Tamil Nadu and Kerala and their morphology and cytology studied.

2. Distribution and morphology

S. nitidum has been reported to be found in the western ghats of south India at elevations of 1000–7000 ft. It has been found to thrive best in places with annual rainfall of 60–100 inches (Rangaswami Ayyangar and Ponnaiya 1941).

Based on the available information, a special germplasm collection mission to the western ghats in Tamil Nadu and Kerala states of south India was organized by ICRISAT in collaboration with the National Bureau of Plant Genetic Resources (NBPGR), New Delhi and the Tamil Nadu Agricultural University, Coimbatore. Two panicle samples were collected and their locations are shown in table 1.

These samples were brought to the ICRISAT Center and they were grown initially in the glass house and subsequently transplanted in the ICRISAT Botanical Garden during 1988 rainy season. One of the plants which has established, flowered

Table 1. Location particulars of *S. nitidum* panicle samples collected from western ghats, during December 1987.

| Collection | State | District | Village | Exact location | | Altitude |
				Latitude	Longitude	
PMP 48	Tamil Nadu	Anna	Melpallam	10° 20'N	77° 35'E	1450 m
PMP 85	Kerala	Idukki	Bodimetti	10° 10'N	77° 15'E	1300 m

and set seed was identified as *S. nitidum* by its distinguishing morphological characters discussed below.

S. nitidum collected in the original habitat was a perennial without any rhizomes spreading in patches on the mountaneous slopes with sparse seed setting. The plant that survived (PMP 85) in the ICRISAT Botanical Garden from the seed of the two accessions collected tillered profusely, spread horizontally on the ground in all directions with thin culms and narrow leaves. Nodes of the culms were bearded; ligule hairs prominent; primary branches of the panicle whorled and simple; racemes terminal (figure 1); peduncles very slender; sessile spikelets small, around 4 mm long and 1·5 mm wide; awns of the sessile spikelet not prominent; pedicelled spikelets around 5 mm long and 1 mm wide, staminate or neuter, lacking lemmas; mature caryopsis abovoid. Seed setting in the plant was also very sparse, just like the plants in the original habitat.

3. Cytology

Meiosis was studied in pollen mother cells (PMCs). Young inflorescences from the surviving plant were fixed in Carnoy's solution for 24 h and stored in 70% alcohol until examined. The anthers were dissected from the spikelets and smeared in acetocarmine (1%), and photomicrographs were taken from temporary slide preparations.

Meiotic studies revealed that the species has 10 chromosomes (n = 5). In addition to the standard (A) chromosomes, each PMC also contained 3 supernumerary (B) chromosomes (figures 2 and 3). The B chromosomes were smaller in size and paired only among themselves. At diakinesis and metaphase I, of the 140 PMCs studied, the A chromosomes formed 5 bivalents in 97% of the cells; while 2 univalents and 4 bivalents were recorded in 3% of the cells studied. The B chromosomes remained as univalents in 44·3% of the PMCs, however, in 55·7% of the cells two of the three B chromosomes formed a bivalent. Forty five PMCs were studied at anaphase I. Normal segregation (5:5) was observed in the standard chromosomes, and a 2:1 distribution of the B chromosomes was recorded in a majority of the cells. Abnormalities like delayed segregation, bridge formation and division of B chromosomes were observed in 24·4% of the anaphase I cells. Pollen fertility of the plant as observed by stainability with acetocarmine was 50·8%.

In *S. nitidum*, the somatic chromosome numbers 2n = 10, 20 and 40 were observed earlier (Garber 1950; Krishnaswamy and Raman 1953; Celarier 1958; Wu 1978; Gu *et al* 1984). The diploid (2n = 10) and tetraploid (2n = 20) forms of the species, morphologically similar except in plant height (Krishnaswamy *et al* 1956), were reported to occur in India. One of the two accessions collected from the western
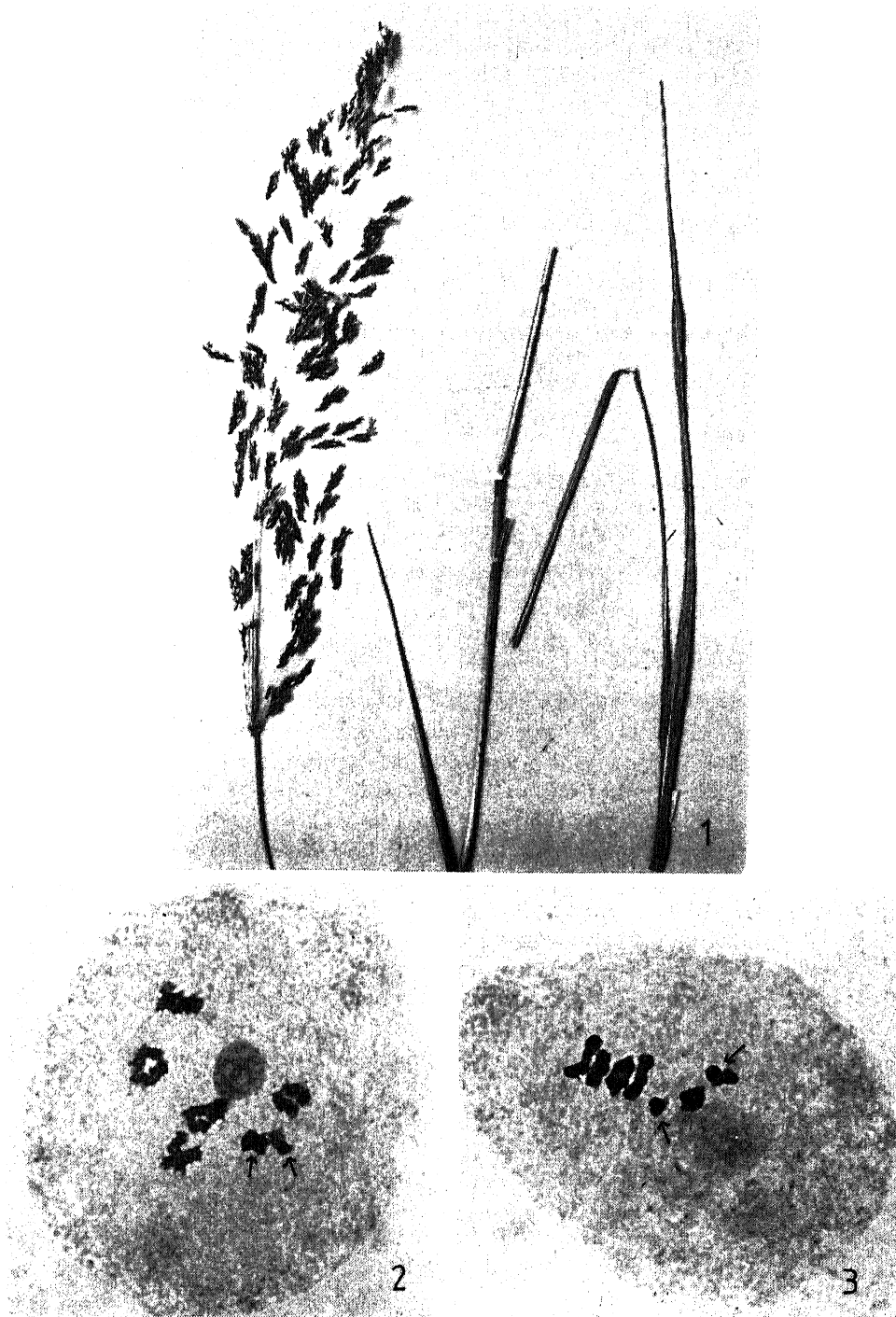
Figures 1–3 1. *S. nitidum* showing panicle branching, bearded sheath nodes and ligule hairs. **2 and 3.** B chromosomes (arrows) at meiosis in *S. nitidum* (× 990). **2.** Diakinesis showing 5 A bivalents + 1 B bivalent + 1 B univalent. **3.** Metaphase I showing 5 A bivalents + 1 B bivalent + 1 B univalent.

ghats now studied has 2n = 10 chromosomes, therefore, it appears that the diploid form is distributed in peninsular India. Although accessory chromosomes were found in *S. nitidum* maintained at Taiwan University, Taipei (Wu 1978), their occurrence in Indian collections is reported for the first time in this paper.

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Effect of domestication on seed packing cost in legumes

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Abstract. The evolutionary shifts in the seed packing features of the plants during domestication were studied by comparing a set of domesticated legumes with the wild species. The domestication considerably reduced the packing cost of the seeds in terms of pod coat weight per unit weight of seeds. This reduction is a result of increased seed weight per pod and the average seed weight, probably at the cost of seed number per pod, while the podcoat weight per pod was maintained during domestication. Eventhough, the average seed weight increased by 8-fold from wild to domesticates, the increase in pod coat weight per seed was only 2–3-fold. It is possible to separate wild and domesticates into distinct clusters on the basis of their individual seed weight and packing costs. We discuss the evolutionary implications of these results.

Keywords. Domestication; seed packing; legumes.

1. Introduction

In the process of domestication, species are subjected to several selective pressures by man for maximization of the productivity (de Wet and Harlan 1975; Harlan 1975). As a result, species have undergone several changes like reduction in number of branches, leaves, internodal length, seed number per pod and seed dormancy; increase in the size of leaf, seed and pod (Harlan *et al* 1973; Kaplan 1965; Morishima *et al* 1963; Smartt 1969, 1976, 1978; Stebbins 1970) and also reduction in pollen to ovule ratio (P/O ratio) (Uma Shaanker and Ganeshaiah 1980).

These changes are generally characterized by an altered resource allocation to the seeds (economic output) from the structures whose importance for survival in the species under domestication is lessened. For instance, reduction in the number of seeds per pod has lead to an increase in size of seeds and pods (Smartt 1969, 1976, 1978). Similar reduction can also be anticipated in the cost of packing the seeds in the pod. Pod coat, being the packing structure also serves several other functions like protection against pests and aids in seed dispersal. Since these functions are not so important under domestication, selection can be expected to reduce the investment on pod coat features. In this paper, we tested this hypothesis in a set of domesticated legumes with their wild relatives. Further, we have also attempted to distinguish the wild and domesticate types on the basis of their pod and seed features like pod coat weight (packing cost) per unit seed weight (P/Sw), packing cost per seed (P/Sn), average seed weight, seed number per pod and pod weight.

2. Materials and methods

The study was conducted on 21 species of wild and domesticated legumes. Mature pods (n = 50) from each species were randomly harvested from their respective

habitats and oven dried. The average pod weight, seed weight per pod, pod coat weight (packing cost) per pod and the seed number per pod were recorded for each species. From this data, the packing cost (pod coat weight) to pack a mg of seed (P/Sw ratio) and the packing cost per seed (P/Sn) were calculated. The mean and variance for wild and domesticates for the above mentioned characters were calculated. Students t-test was used to findout the statistical significance between wild and domesticate species for each character. The P/Sw ratio and average seed weight were transformed to normalized 'Z' values and plotted on a graph (figure 1).

3. Results and discussion

The wild species invest more energy in packing seed (P/Sw = 1·27) than the domesticate species (P/Sw = 0·36) (table 2). For instance, in the genera, *Glycine*, *Macrotyloma* and *Cajanus*, the wild species had 4 times greater P/Sw ratio to their domesticate relatives (table 1).
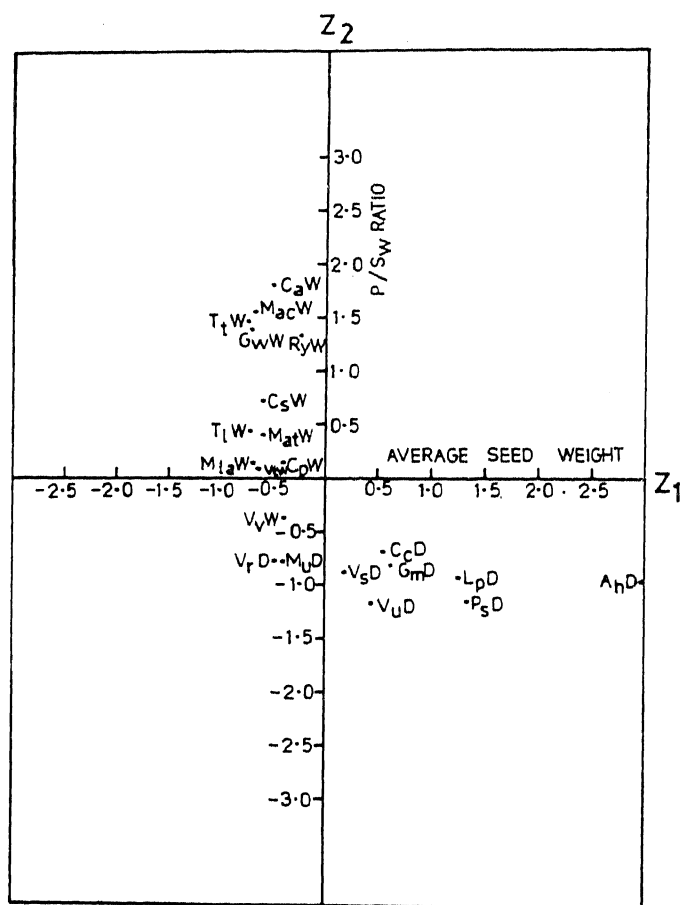
Figure 1. Normalised 'Z' transformed values of average seed weight (Z_1) and P/Sw ratio (Z_2) of wild and domesticate legumes.

Table 1. Comparison of various morphological characters and P/Sw ratio between wild and domesticate legumes.

Species	Abbreviation	Seeds per pod (Sn)	Pod wt. (mg)	Pod coat wt. (P) per pod (mg)	Seed wt. (Sw) per pod (mg)	P/Sw ratio	Average seed wt. (mg)	Pod coat wt. seed (P/Sn ratio) (mg)
1. a. *Macrotyloma accillare*	MacW	7·2	173·3	110·0	63·3	1·74	8·9	15·4
b. *M. uniflorus**	MuD	4·6	240·0	71·3	168·7	0·42	36·3	15·5
2. a. *Glycine wightii*	GwW	4·1	63·0	39·3	23·7	1·66	5·7	9·6
b. *G. max**	GmD	2·3	454·7	133·3	321·4	0·41	142·1	59·0
3. a. *Canjanus albicans*	CaW	4·0	319·3	208·7	110·7	1·88	28·0	52·6
b. *C. scarabeoides*	CsW	4·3	144·3	80·7	63·7	1·27	14·9	18·9
c. *C. cajan**	CcD	4·0	774·3	245·7	528·7	0·46	132·2	61·4
4. a. *Vigna trilobata*	VtW	10·0	193·3	76·7	106·7	0·82	10·7	8·7
b. *V. vexillata*	VvW	12·6	745·7	291·0	454·7	0·64	36·0	23·0
c. *V. radiata**	VrD	11·7	475·7	143·9	331·8	0·43	27·8	12·1
d. *V. umbellata**	VuD	5·6	845·6	149·5	696·1	0·21	124·8	26·7
e. *V. sinensis**	VsD	12·2	1689·5	478·5	1211·0	0·37	96·1	37·9
5. a. *Macroptilium atropurpureum*	MatW	12·2	325·3	166·3	159·0	1·07	13·0	13·9
b. *M. lathyroides*	MlaW	20·3	312·7	140·1	172·6	0·81	8·5	6·9
6. *Centrosema pubescense*	CpW	18·1	1221·0	596·0	625·0	0·95	34·5	32·8
7. *Teramnus labialis*	TlW	9·2	153·3	80·7	72·7	1·11	7·9	8·8
8. *Tephrosia tinctoria*	TtW	8·9	98·3	61·3	37·0	1·66	4·0	7·0
9. *Rynchosia viscera*	RvW	2·0	265·0	163·3	101·7	1·61	51·9	83·4
10. *Lab-lab purpureus**	LpD	3·6	977·0	247·7	729·3	0·34	201·9	68·7
11. *Arachis hypogea**	AhD	1·3	720·8	183·3	537·5	0·34	422·0	144·1
12. *Pisum sativum**	PsD	3·4	847·5	153·6	695·4	0·22	213·4	46·7

*Average of 3–4 varieties.
W, Wild species; D, domesticate species.

Table 2. Comparison of mean, standard deviation and range between wild and domesticates for various morphological characters.

Character	Wild		Domesticates		Significance (P<)
	Mean ± SD	Range	Mean ± SD	Range	
Seed No. per pod	9·4 ± 5·5	2·0–20·3	5·4 ± 3·7	1·3–12·2	0·05
Pod weight (mg)	334·5 ± 316·8	63·0–1221·0	780·6 ± 389·9	240·0–1689·5	0·05
Pod coat weight per pod (mg) (packing cost)	168·3 ± 145·4	39·3–596·0	200·8 ± 111·1	71·3–478·5	NS
Seed weight per pod (mg)	165·9 ± 175·9	23·7–625·0	580·1 ± 288·7	168·7–1211·0	0·01
P/Sw ratio	1·27 ± 0·41	0·64–1·88	0·36 ± 0·08	0·21–0·43	0·01
Average seed wt. (mg)	18·7 ± 14·6	4·0–51·9	155·3 ± 111·6	27·8–422·0	0·01
Pod coat weight per seed (mg)	23·4 ± 22·1	6·9–83·4	52·4 ± 37·5	12·1–144·1	0·05
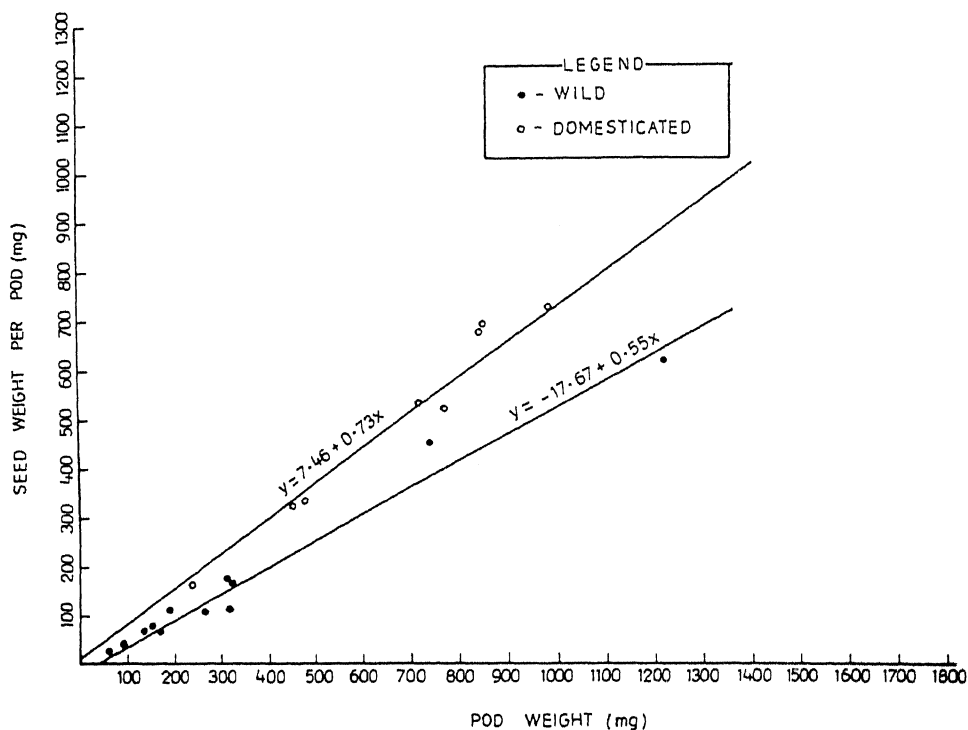
Figure 2. Relationship between pod weight and seed weight per pod in wild and domesticate legumes.

Such decrease in P/Sw ratio during domestication probably indicates a reallocation of resources to the seed, increasing the seed size (Wells 1976). This is evident from the data on pod weight, pod coat weight (packing cost) and seed weight per pod (table 2). The mean pod weight (780 mg) of domesticates was 2-fold more than the wild types (334·5 mg). On the other hand, the increase in the total

pod coat weight was negligible (200·8 mg in domesticates and 168·3 mg in wild). As a result, the average seed weight increased 8-fold from wild (18·7 mg) to domesticate (153·3 mg) probably to an extent at the cost of seed number per pod, while pod coat weight per seed increased by only 2-3-fold (23·4 mg in wild and 52·4 mg in domesticates). Hence the increase in pod weight and decrease in P/Sw ratio from wild to domesticates is mainly due to the increased seed weight, while the pod coat weight was unaltered.

The regression co-efficients indicate, that for every mg increase in pod weight, in domesticates 0·73 mg increase in seed weight and only 0·27 mg increase in pod coat weight was recorded, whereas in wild species it was only 0·55 mg increase in seed weight and an increase in pod coat weight as high as 0·45 mg (figure 2). This suggests that comparitively wild species invest more on pod coat than on seeds. Further, for every mg increase in average seed weight, the wild species invests as much as 1·34 mg in pod coat weight whereas in domesticates it is only 0·31 mg (figure 3).

Such shifts could be due to the selective pressures in domestication, where survival strategies of wild species have been rescheduled to suit the domesticate habitats. For instance, increase in seed size is an important determinant of seedling establishment (Harper 1977; Marshal 1986), and is highly preferred under domesticated conditions (de Wet and Harlan 1975). Infact, this is a general feature of pulses, where seed size is shown to have significant increase in all cultigens *vis a*
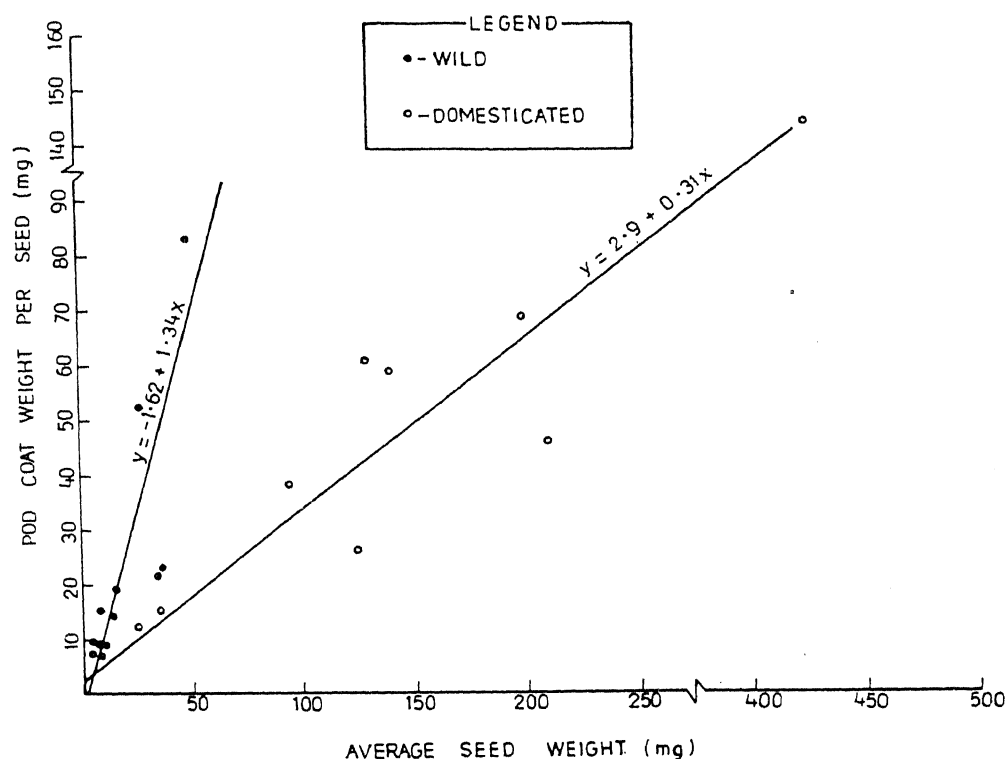
Figure 3. Relationship between average seed weight and pod coat weight per seed of wild and domesticate legumes.

vis their wild relatives (Smartt 1976). This imposes an altered reproductive strategy, resulting in fewer but larger propagules under domestication which is evident from the present study. Further, it is well established that species with small seeds tend to occupy habitats that are more sunny, dry and disturbed, while plants species of stable, shady and moist habitats generally have larger seeds (Baker 1972; Primack 1987; Salisbury 1942). Thus, wild species fall into former habitats with small and more number of seeds per pod and domesticate species into later habitats with larger and less number of seeds per pod. Also, under explosive dispersal (dehiscence of pods), smaller seeds can be dispersed to larger distances which is an added advantage for wild species. The torsion force and the tension required for such sudden and violent pod dehiscence is by increased lignification of the parchment layer fibres. This is accomplished in wild species by increased pod coat weight.

In order to distinguish wild and domesticate species, Gentry (1969) and Smartt (1969, 1978) used several morphological characters, while Uma Shaanker and Ganeshaiah (1980, 1982) used pollen to ovule ratio. Similarly P/Sw ratio coupled with average seed weight may be reasonably used as an indicator of the species habitat. In fact by transforming the values of these two parameters into normalized 'Z' values and plotting on a graph, we could seggregate the wild and domesticate species into the diagonally opposite quadrants (figure 1).

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Nitrogen fixation by *Candida tropicalis*

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Abstract. Several strains of *Candida tropicalis* isolated from the dung of buffalo, cow and goat have been found to fix N_2. The nitrogenase activity (acetylene reduction) varied between 73 and 1162 nmol (mg protein)$^{-1}$ h^{-1}. 2·2–9·3 mg N_2 were fixed g^{-1} glucose consumed. Acetylene was reduced progressively with time. Acetylene reduction could not be observed in cells treated with cycloheximide but was unaffected by antibiotics which inhibit the growth of prokaryotes.

Keywords. *Candida tropicalis*; N_2 fixation; animal dung.

1. Introduction

During an investigation on the occurrence of N_2-fixing microorganisms in the faeces of animals, particularly cattle dung, which is used extensively as a manure in this part of the world, we detected the presence of yeast cells capable of fixing N_2 in laboratory media. N_2 fixation by several yeasts like *Torula wisneri*, *Pullularia* sp. and several species of *Saccharomyces* has been claimed (see Mishustin and Shil'nikova 1971, for a discussion). Such claims could not be substantiated by Millbank (1969, 1970) and Postgate (1979), and no yeast figures in the list of N_2-fixing microorganisms of Postgate (1982). Line and Loutit (1973) examined the probable causes of failure of oligonitrophilic organisms to fix N_2 and observed that microorganisms which do not fix N_2 by themselves may do so in syntrophic association with an N_2-fixing microorganism, particularly anaerobes like *Clostridium*, due to development of reduced conditions in liquid enrichment cultures by the respiratory activity of the non-dinitrogen-fixing component. Stimulatory effects of *Saccharomyces* and *Rhodotorula* on N_2 fixation by *Azotobacter* have been reported (Fedorov 1960; Mulder *et al* 1969). Jensen and Holm (1975) however, found no stimulatory effect of *Candida curata*, *Torulopsis acria* and *Lipomyces starkeyii* on N_2 fixation by a bacterium N 63 which had considerable resemblance to *Derxia*. According to Hill and Postgate (1969) several non-dinitrogen fixers are also scavengers of traces of fixed nitrogen present in the laboratory air as ammonia or nitrogen oxides or nitrogenous impurities present in the N-free culture media.

Babeva *et al* (1977) have reported that the soil yeast *Lipomyces lipofer* 133 which by itself has no detectable nitrogenase activity, increases N_2 fixation by *Pseudomonas* sp5 (the purity and identity of which has not been confirmed) about 15-fold. The possibility of induction of nitrogenase activity in *Lipomyces* by some metabolite of *Pseudomonas* or by the microaerophilic condition obtaining in such an association, as it happens in the case of induction of nitrogenase activity in *Rhizobium ex planta* (see Robson and Postgate 1980), has not been investigated.

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Dommergues and Mutaftschief (1965) have observed that N_2 fixation by *Beijerinckia indica* and *B. fluminensis* two aerobic N_2-fixers, was strongly stimulated by a soil yeast *L. starkeyii*. *L. starkeyii* is used in the lipid industry and surprisingly grows well even when the medium-N is almost exhausted. N_2 fixation by 'pure' cultures of *L. starkeyii* has been claimed recently by Samanta *et al* (1983) and Sen (1982).

We have so far isolated 13 strains of yeasts from the dung of herbivorous domestic animals, cow, buffalo and goat, which were found to fix N_2. In view of the observations recorded above, it was necessary to purify the cultures since true eukaryotic N_2 fixation is disputed, using rigorous tests, as far as possible.

2. Materials and methods

2.1 *Isolation*

The organisms were isolated from dung samples collected from different places in West Bengal by the enrichment method. One gram wet weight of the samples was transferred to 9 ml sterile water, shaken vigorously and 1 ml transferred to Burk's N-free DN_2 medium. At weekly intervals 1 ml of the culture was transferred to fresh Burk's N-free DN_2 medium and the operation continued for 7 weeks. Samples enriched this way were subjected to dilution plating in the same N-free medium. The isolated colonies were then transferred to N-free broth containing 10, 25, 50 and 100 $\mu g\, ml^{-1}$ each of penicillin, streptomycin, terramycin and chloramphenicol. The surviving cells were then subjected to dilution plating and the colonies transferred to N-free broth containing a mixture of all these antibiotics mentioned to remove contaminant cells, if any, which may be resistant to any of these antibiotics. The contents were then again dilution plated and the colonies transferred to N-free medium containing 200 $\mu g\, ml^{-1}$ each of kanamycin and ampicillin separately and in combination and subjected to dilution plating. The surviving colonies were then grown on Waksman's acid agar (pH 3·8) which permit the growth of yeasts but not most bacteria, followed by dilution plating. The purified cultures were maintained on Burk's DN_2 medium containing 0·025% yeast extract for supplying the growth factors required for good growth and a small amount of starter N, which was useful for their growth.

2.2 *Media*

Burk's N-free DN_2 medium had the following composition (g l^{-1}): D-glucose 20; $MgSO_4$, $7H_2O$ 0·02; K_2HPO_4 0·2; KH_2PO_4 0·8; NaCl 0·2; $CaCl_2 \cdot 2H_2O$ 0·09; Fe-Mo solution ($FeSO_4 \cdot 7H_2O$ 500 mg, $Na_2\, Mo\, O_4$, $2H_2O$ 2·52 mg in 100 ml distilled water). Yeast extract when required was added at 0·025%.

Waksman's acid agar contained (g l^{-1}): D-glucose 10; KH_2PO_4 1; $MgSO_4 \cdot 7H_2O$ 0·5; Peptone 0·5; agar 20; pH 4.

2.3 *Scanning electron microscopy*

For scanning electron microscopy washed cells were treated with 3% glutaraldehyde

plus 5% dimethyl sulphoxide for 10 min followed by centrifugation; the cells were washed with 50 mM sodium acetate buffer, centrifuged and treated with 0.02 mM OsO_4 for 10–15 min until a black colour appeared. Distilled anhydrous acetone was used for dehydration of the cells on a glass plate and finally sputtered with gold for observation with a Philips scanning electron microscope 500 at the Regional Sophisticated Instrumentation Centre, Bose Institute, Calcutta.

2.4 *Biochemical characteristics*

The biochemical characteristics of the strains were examined according to Kreger-Van Rij (1969) and Gentles and La Touche (1969).

2.4a Nitrogen estimation: Nitrogen content of the cultures was estimated by the microkjeldahl method using a Markham and Smith still. Ten ml samples of 7-day-old cultures grown on N-free DN_2 medium without yeast extract was taken in microkjeldahl flasks and digested with 50% H_2SO_4, a pinch of K_2SO_4 and 1 ml 20% $CuSO_4$ until the solution was clear. The digest was then treated with 30% NaOH containing 5% $Na_2S_2O_3$ and steam distilled. The distillate was collected in N/50 HCl and titrated with N/50 NaOH using Weslow's indicator. Samples of uninoculated medium, subjected to identical incubation conditions for 7 days, were also analysed for N and deducted from those obtained with inoculated cultures.

2.4b Protein estimation: Protein was estimated following the method of Lowry *et al* (1951).

2.4c Acetylene reduction: For acetylene reduction studies 7-day-old cultures of yeast cells grown on Burk's DN_2 medium containing 0.025% yeast extract was centrifuged in stoppered sterile centrifuge tubes at 4,400 g and washed twice with sterile Burk's N-free DN_2 medium without yeast extract. The pellet was transferred to fresh Burk's N-free DN_2 medium and incubated for 24 h at 30°C. One ml culture containing 2×10^7 cells was then transferred to 8 ml vials; the vials were evacuated and argon and acetylene introduced to provide a concentration of 10% acetylene and 90% argon, the pressure inside being 1.0 atmos. Incubation temperature was 30°C. One ml samples of the vial atmosphere were removed at periodic intervals corresponding to different points of growth phase and injected into a Porapak N-column (Field Instrument Co., England) fitted into a Hewlett Packard 5730 A gas chromatograph having a hydrogen flame ionization detector. During acetylene reduction the oven temperature was 80°C and the detector temperature was 100°C. Nitrogen was used as the carrier gas.

3. Results

3.1 *Purity of cultures*

The strains were isolated from the dung samples by the enrichment method followed by dilution plating and purification through the use of several antibiotics as described under 'Materials and methods'. All the 13 strains isolated this way

survived treatment with the antibiotics tested. Four of these strains KUB_1, KUB_6, KUC_{45} and KUG_6 were selected for a detailed study. The antibiotic sensitivity of these 4 strains is shown in table 1. When *Klebsiella pneumoniae* M_5 al (a strain obtained from J R Postgate, of the University of Sussex, England) could not grow in the presence of 200 μg ml^{-1} of the antibiotics, the strains of the yeasts examined had no difficulty for growth. Cycloheximide, an inhibitor of eukaryotic protein synthesis however, at a concentration of 1000 μg ml^{-1} was lethal to all the 4 strains although *K. pneumoniae* M_5 al was unaffected (table 1). No growth was also observed when cycloheximide (1000 μg ml^{-1}) treated cells were incubated with nutrient broth, nutrient agar and Burk's DN_2 medium with and without 0·025% yeast extract under both aerobic and anaerobic condition; but *K. pneumoniae* continued to grow actively in most of these media. If the diazotrophic yeast cells were associated with any prokaryotic non-N_2-fixer then no nitrogenase activity would be expected in their cultures if pretreated with 1000 μg ml^{-1} cycloheximide. Table 2 shows that nitrogenase activity as indicated by acetylene reduction could not be detected in any of the yeasts treated with cycloheximide; however, *K. pneumoniae* treated identically with cycloheximide continued to reduce acetylene actively.

Further evidence that the strains were not associated with N_2-fixing bacteria were provided in an experiment in which the yeast cells were grown in Waksman's acid agar. Not only that the strains grew well at acidic pH in successive subcultures the optimum pH for growth of these yeast cells was found to be 4·6. Acetylene was reduced even at this pH (Saha and Sen 1990).

The strains isolated reduced acetylene progressively with time with a lag period of 1–3 h (figure 1). There was some parallelism between growth and nitrogenase activity. The strains were most active during the exponential phase of growth but some activity was also detected at 24 h when the cells had reached the stationary phase. If the nitrogenase activity observed is entirely due to contaminant cells, then samples withdrawn at late exponential or stationary phase of growth should reveal the presence of large number of bacterial cells. In none of the experiments with any of the yeast cultures such evidence was obtained. Phase contrast, fluorescence and

Table 1. Growth ($A_{540\,nm} \pm SE$) of different yeast isolates treated with antibiotics (200 μg ml^{-1}) as compared to that of *K. pneumoniae* M_5al. The cultures were incubated at 30°C and growth was measured after 24 h.

Laboratory Index No.	Control	Ampicillin	Penicillin	Streptomycin	Chloramphenicol	Tetracycline	Kanamycin	Cycloheximide (1000 μg ml^{-1})
KUB_1	0·34±0·01	0·21±0·01	0·16±0·01	0·15±0·01	0·15±0·01	0·18±0·01	0·12±0·01	0·0
KUB_6	0·37±0·01	0·32±0·01	0·29±0·01	0·20±0·01	0·28±0·01	0·31±0·01	0·15±0·01	0·0
KUC_{45}	0·35±0·003	0·12±0·01	0·12±0·01	0·12±0·01	0·15±0·00	0·17±0·01	0·12±0·003	0·0
KUG_6	0·35±0·003	0·25±0·01	0·21±0·01	0·17±0·01	0·15±0·00	0·17±0·01	0·12±0·003	0·0
K. pneumoniae M_5al	0·25±0·01	0·00	0·002±0·00	0·03±0·00	0·0	0·0	0·04±0·00	0·25
CD at								
$P=0·01$	0·0019	0·004	0·0036	0·0029	0·0021	0·0280	0·0014	0·001
$P=0·05$	0·0012	0·0026	0·0023	0·0019	0·0013	0·0018	0·0009	0·0016

Table 2. Effect of cycloheximide $(100\ \mu g\ ml^{-1})$ and ampicillin $(200\ \mu g\ ml^{-1})$ on acetylene reduction by strains of yeasts and *K. pneumoniae* M_5 al ±SD.

Laboratory Index No.	Treatment	n-mol ethylene formed mg^{-1} protein h^{-1} ±SD
KUB$_1$	—	608·0 ± 6·96
KUB$_1$	Cycloheximide	0·0
KUB$_6$	—	661·0 ± 4·93
KUB$_6$	Cycloheximide	0·0
KUC$_{45}$	—	510·66 ± 5·20
KUC$_{45}$	Cycloheximide	0·0
KUG$_6$	—	1054·33 ± 33·09
KUG$_6$	Cycloheximide	0·0
K. pneumoniae M_5 al	—	911·66 ± 6·00
K. pneumoniae M_5 al	Cycloheximide	710·0 ± 8·66
KUB$_1$	Ampicillin	478·33 ± 17·40
KUB$_6$	Ampicillin	603·33 ± 12·01
KUC$_{45}$	Ampicillin	416·66 ± 22·04
KUG$_6$	Ampicillin	558·33 ± 22·04
K. pneumoniae M_5 al		0·0
CD $P = 0·01$		48·71
CD $P = 0·05$		42·36

The microbial cultures were grown on Burk's DN_2-agar slants containing 0·025% yeast extract for 48 h. The growth was aseptically scraped off the surface and suspended in sterile. Burk's N-free broth without yeast extract for 6 h at 30°C; 0·9 ml of the suspension (OD 0·36 at 540 nm) was then transferred to sterile 8 ml vials followed by the addition of 0·1 ml of cycloheximide (10 mg ml^{-1}) or ampicillin (2 mg ml^{-1}) and incubated at 30°C for 24 h with 10% acetylene in argon atmosphere for assay of nitrogenase activity.

electronmicrographs also did not show the presence of any bacteria associated with the yeasts. Some representative isolates are shown in figure 2.

3.2 *Morphological characteristics of the culture*

Cells are round to oval, occurring singly or in groups, $4–8 \times 4–10\ \mu m$. In broth cultures by 3 weeks lumpy or flocculent deposit or a few islands of a milky film are formed. Streak cultures on agar slopes give white shiny, creamy or dull smooth colonies. Under aerobic conditions on potato dextrose agar well developed pseudomycelium with blastospores occurring singly or in clusters are detected; under anaerobic conditions true mycelium is produced, some unbranched, some with short branches with blastospores. On corn meal agar under aerobic conditions long unbranched true mycelium is produced with some pseudomycelia and occasional cells; mycotorula are also produced in some cases.
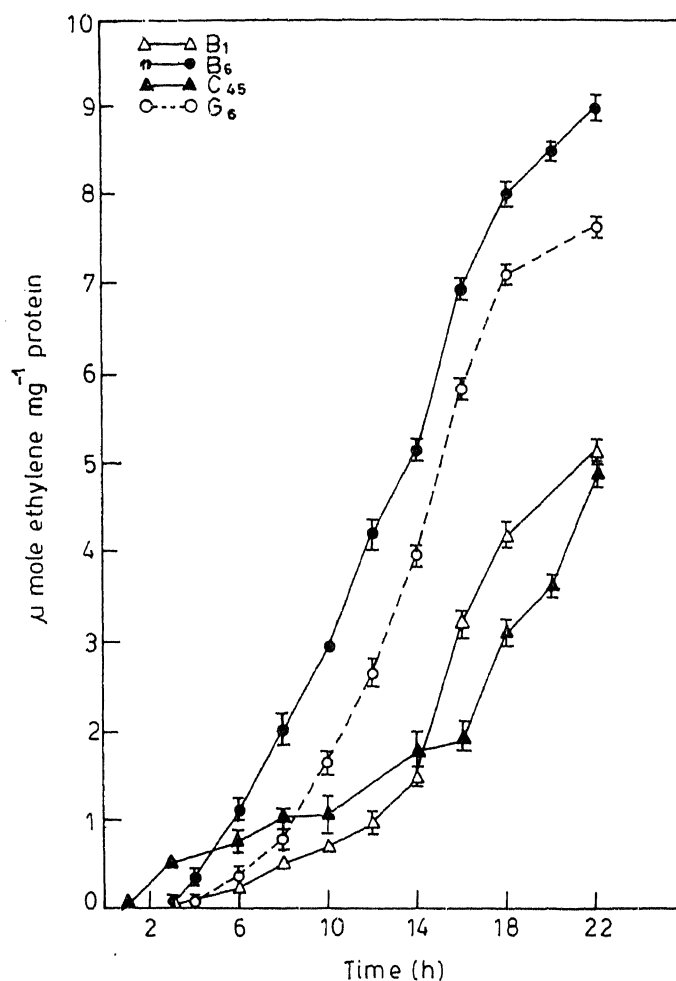
Figure 1. Time course curve for acetylene reduction by 4 yeast isolates.

3.3 *Biochemical characteristics*

Glucose and fructose are utilized most actively by all the 4 strains; melibiose, raffinose, cellobiose, inulin and xylose are not fermented by any of these strains. L-rhamnose, glycerol, galactitol, inositol, methanol, and glucosamine are not assimilated. Ammonium sulphate and ethylamine hydrochloride are utilized, and fat and arbutin are hydrolyzed in 21 days. Growth occurs in vitamin free medium and even in the presence of 10% NaCl.

The strains fix 18–69 mg $N_2 \, l^{-1}$-culture medium as estimated by the microkjeldahl method; $2 \cdot 2$–$9 \cdot 3$ mg N_2 are fixed g^{-1} of glucose consumed. Acetyline reducing capacity of the strains vary between 73 and 1162 nmol mg^{-1} protein h^{-1} (table 3).

Three of these strains have been identified as *Candida tropicalis* by the National Collection of Yeast Cultures, Norwich, UK, who assigned the numbers NCYC 1523, 1524 and 1525 to the strains KUB_1, KUB_6 and KUC_{45}, respectively. The
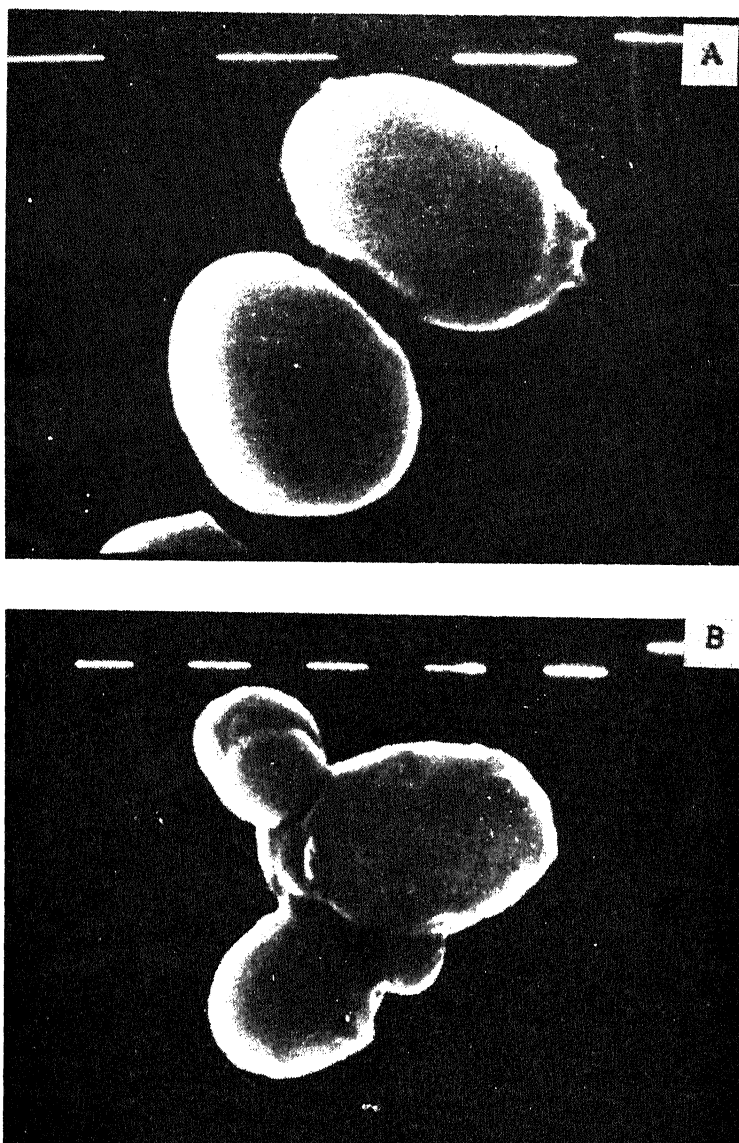
Figure 2. Scanning electron micrographs of yeast isolates. (A) KUB_1. (B) KUB_6. The bar represents 1 μm.

remaining 10 cultures have very ·similar morphological and biochemical characteristics.

4. Discussion

The tolerance of high concentrations of several broad spectrum antibiotics, singly and in mixture, the lack of nitrogenase activity in the presence of 1000 μg ml^{-1}

Table 3. Nitrogen-fixing capacity of yeast cells isolated from the dung samples of domestic animals.

Source	Index No.	N_2 fixed (mg)		nmol ethylene formed mg^{-1} protein $h^{-1} \pm SD$
		l^{-1}	g^{-1} glucose consumed	
Buffalo	KUB_1	$69 \cdot 33 \pm 1 \cdot 45$	$8 \cdot 60 \pm 0 \cdot 11$	$454 \cdot 30 \pm 5 \cdot 36$
	KUB_3	$51 \cdot 70 \pm 0 \cdot 06$	$7 \cdot 18 \pm 0 \cdot 01$	$182 \cdot 60 \pm 1 \cdot 45$
	KUB_6	$66 \cdot 00 \pm 0 \cdot 00$	$8 \cdot 58 \pm 0 \cdot 03$	$1162 \cdot 00 \pm 6 \cdot 11$
	KUB_7	$41 \cdot 75 \pm 0 \cdot 02$	$3 \cdot 48 \pm 0 \cdot 03$	$311 \cdot 00 \pm 2 \cdot 08$
	KUB_8	$55 \cdot 69 \pm 0 \cdot 01$	$7 \cdot 96 \pm 0 \cdot 09$	$371 \cdot 30 \pm 5 \cdot 92$
Cow	KUC_{10}	$40 \cdot 32 \pm 0 \cdot 02$	$2 \cdot 69 \pm 0 \cdot 01$	$569 \cdot 66 \pm 3 \cdot 68$
	KUC_{22}	$20 \cdot 16 \pm 0 \cdot 03$	$2 \cdot 24 \pm 0 \cdot 01$	$117 \cdot 43 \pm 2 \cdot 04$
	KUC_{31}	$22 \cdot 88 \pm 0 \cdot 01$	$3 \cdot 58 \pm 0 \cdot 01$	$110 \cdot 00 \pm 4 \cdot 08$
	KUC_{41}	$17 \cdot 67 \pm 0 \cdot 12$	$2 \cdot 39 \pm 0 \cdot 01$	$72 \cdot 76 \pm 2 \cdot 07$
	KUC_{45}	$66 \cdot 50 \pm 0 \cdot 28$	$9 \cdot 30 \pm 0 \cdot 08$	$463 \cdot 33 \pm 12 \cdot 47$
Goat	KUG_2	$29 \cdot 04 \pm 0 \cdot 29$	$2 \cdot 91 \pm 0 \cdot 02$	$249 \cdot 66 \pm 5 \cdot 78$
	KUG_6	$40 \cdot 60 \pm 0 \cdot 20$	$3 \cdot 31 \pm 0 \cdot 02$	$582 \cdot 20 \pm 6 \cdot 17$
	KUG_{21}	$29 \cdot 37 \pm 0 \cdot 12$	$2 \cdot 94 \pm 0 \cdot 01$	$189 \cdot 33 \pm 3 \cdot 48$

cycloheximide, growth in media having pH values of 4·6 or lower, absence of any detectable contaminant cells at any stage of growth as revealed by optical, phase contrast, fluorescence and scanning electron microscopy strongly suggest that the strains of yeasts isolated are pure cultures. The absence of growth and N_2 fixation in the presence of cycloheximide under anaerobic conditions also preclude the possibility of the presence of *Clostridium* and other syntrophic anaerobic N_2-fixers. Among the clostridia which occur in animal faeces *C. butyricum* has been reported to fix N_2. Unlike the strains of yeasts used, *C. butyricum* does not grow on nutrient agar and cannot utilize sorbitol and melezitose. *C. pasteurianum* and *C. acetobutylicum* which occur in ocean and in soil and fix N_2 also differ from the yeasts in their capacity to utilize several carbon sources. Most N_2-fixing bacteria cannot grow and fix N_2 at acidic pH with the exception of *Beijerinckia*, *Derxia*, *Xanthobacter flavus* and *Azomonas macrocytogenes*. *Beijerinckia* and *Derxia* produce exceptionally gummy colonies, *X. flavus* does not use sugars and *A. macrocytogenes* grows in water and produces a blue pigment, which was never detected. The guanosine + cytosine content of the strains of *C. tropicalis* studied here as determined from the melting temperature of the DNA is 57·6 mol per cent against 26·28 mol per cent for the N_2-fixing strains of *Clostridium*. Although this is rather close to the range of the G + C contents of DNA of *Klebsiella* (52–56 mol%), the experiments with antibiotics clearly preclude the presence of *Klebsiella* in association with the yeast strains studied. G + C content of *Azotobacter* DNA varies between 63 and 66 mol per cent (Bergey 1975).

The lag phase of 1–3 h observed for N_2 fixation by yeast cells is not surprising, since the yeast cells also exhibit largely similar lag phases in their growth as was also shown by Khan and Sen (1974) for *Saccharomyces* and *Candida*. Samples of yeast cultures removed at different points of exponential growth phase when transferred to an acetylene atmosphere exhibited similar lag periods before acetylene reduction commenced. The yeast cells may also take some time to adapt themselves to an atmosphere of acetylene.

C. tropicalis has been reported to occur in soil, grains and digestive tracts and infected tissues of animals, including man (Kawakita and Van Uden 1965; Do Carmo-Souza 1969; Gentles and La Touche 1969). Various yeast cells including other species of *Candida* have been reported to occur on the leaf surface of plants (Ruinen 1971; Do Carmo-Souza 1969) and may find their way into the rumen after the plants are consumed. In view of the observations of Zarmır *et al* (1981) that the *nif* gene of *K. pneumoniae* transferred to *Saccharomyces cerevisiae* is not functional even though the gene is integrated with the genome of the recipient, our observation that several strains of *C. tropicalis* can fix nitrogen, indicates that the *nif* gene has been stabilized and is functional in this eukaryotic cell, opening up the possibility of its use, in genetic engineering studies. It has been suggested (Cocking 1981) that the *nif* gene of *Klebsiella* is probably not transcribed by the eukaryotic RNA polymerase and the nitrogenase produced is inactivated by O_2. The strain KUB_6 or *C. tropicalis* appears to be less O_2-sensitive than several N_2-fixing microorganisms (Saha 1987); possibly a more extensive search may bring to light the existence of better strains with lower sensitivity to O_2.

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Nitrogenase activity of diazotrophic strains of *Candida tropicalis*

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Abstract. Nitrogenase activity of 4 strains of *Candida tropicalis* exhibited a pH optimum of 7·2 and an optimum temperature of 30°C; it was depressed by NH_4^+ ions and at 1 mM no activity could be detected. 2,4-Dinitrophenol, sodium azide and *p*-chlormercuribenzoate abolished nitrogenase activity completely at $2·5 \times 10^{-4}$ M. Nitrogenase activity was increasingly inhibited by O_2 with increasing concentrations; it was also inhibited by methane and methanol. The yeast cells possessed active uptake hydrogenase activity.

Keywords. *Candida tropicalis*; Nitrogenase activity; O_2 sensitivity; NH_4^+ repression; uptake hydrogenase activity.

1. Introduction

We have reported in the previous paper (Saha and Sen 1990) the occurrence of N_2-fixing yeast cells in the dung samples of buffalo, cow and goat. On the basis of morphological and biochemical characteristics these strains were identified as *Candida tropicalis*. In this paper we summarize our observations concerning nitrogenase activity of these diazotrophic yeast cells.

2. Materials and methods

The cultures used were KUB_1, KUB_6, KUC_{45} and KUG_6 isolated from buffalo, cow and goat, respectively. The isolation, purification and characterisation of these microorganisms as also the optimum conditions of growth and composition of culture media have been described in the previous paper (Saha and Sen 1990).

Nitrogenase activity was assayed by the acetylene reduction method as described previously. To study the effect of temperature on nitrogenase activity 1 ml of culture ($A_{540\,nm}$ 0·36) suspended in Burk's N-free DN_2 broth (pH 7·2) was incubated with 10% acetylene in argon for 24 h in sealed vials at different temperatures and acetylene reduction was studied at periodic intervals. For pH effects the same procedure was followed except that the pH of the incubation mixture was varied by inclusion of buffers of appropriate pH range.

To test O_2 sensitivity of nitrogenase, known volumes of O_2 (Indian Oxygen Co., Calcutta) were injected into the experimental vials to provide different concentrations of O_2. To study the effect of methane the same procedure was followed except that known volumes of biogas containing 2·5 µmol of methane ml^{-1} were introduced into the vials alongwith acetylene and argon.

NH_4^+-repression of nitrogenase activity was studied in two different ways. In one set of experiments different concentrations of NH_4Cl were added at the

commencement of the experiment and in the other set NH_4^+ was introduced 30 min after the commencement of acetylene reduction. Samples were withdrawn after 4, 5, 6, 8 and 12 h of incubation. The control set in each case received the same volume of distilled water. The effect of the following inhibitors was also studied: 2, 4-dinitrophenol, sodium azide and *p*-chlormercuribenzoate.

To study hydrogenase and uptake hydrogenase activity, the H_2 content of the vials was measured with a Hewlett Packard gas chromatograph fitted with a thermal conductivity detector. To study uptake hydrogenase activity, known volumes of H_2 were introduced into the vials. Samples were taken periodically for 50 h.

3. Results

Nitrogenase activity of the yeasts was influenced by both temperature and pH. The optimum temperature was found to be 30°C and higher temperatures were distinctly inhibitory (table 1). At 30°C the optimum pH was found to be 7·2 (table 2). At pH 4·6 and 9·2 the nitrogenase activity was only about 10% of the optimum value. However, as the same ingredients could not be used for preparation of buffers of different pH values, the effect of different ingredients on nitrogenase activity cannot be entirely ruled out.

As in most diazotrophs nitrogenase activity was found to be repressed by NH_4^+. In KUB_6 NH_4Cl at all concentrations used, inhibited nitrogenase activity, the inhibition increasing with increasing NH_4^+ concentrations. With 1 mM NH_4^+ no nitrogenase activity could be detected at 4 h of incubation, after 5 h the same was also observed with 5 mM NH_4^+ (table 3). Slow recovery was noted after 4 h with

Table 1. Effect of temperature on nitrogenase activity of the yeast isolates KUB_6 and KUC_{45}.

Temperature (°C)	nmol acetylene reduced mg^{-1} protein h^{-1}	
	KUB_6	KUC_{45}
8	0	0
30	336·65 ± 5·18	285·65 ± 4·82
35	210·82 ± 4·75	150·42 ± 6·85
42	0	0

Table 2. Effect of pH on nitrogenase activity of the yeast isolate KUB_6.

pH	nmol acetylene reduced mg^{-1} protein h^{-1}
4·6	99·0 ± 1·82
5·7	115·5 ± 3·62
6·7	264·0 ± 5·74
7·2	825·0 ± 3·36
8·0	198·5 ± 3·25
9·2	66·0 ± 1·36

Table 3. Effect of NH_4Cl on nitrogenase activity (acetylene reduction) of KUB_6.

Concn. of NH_4Cl (mM)	nmol of acetylene reduced				
	Time of incubation (h)				
	4	5	6	8	12
0	27·87 (100)	222·97 (100)	557·44 (100)	2229·36 (100)	7135·23 (100)
1	0	167·23 (75)	445·90 (80)	2006·78 (90)	6689·28 (93)
2	0	111·48 (50)	334·46 (60)	1672·32 (75)	6243·32 (87)
3	0	111·48 (50)	334·46 (60)	1560·83 (70)	4459·52 (62)
5	0	0	222·97 (40)	1114·80 (50)	3344·64 (46)

Numbers in parentheses are percentage of control values.
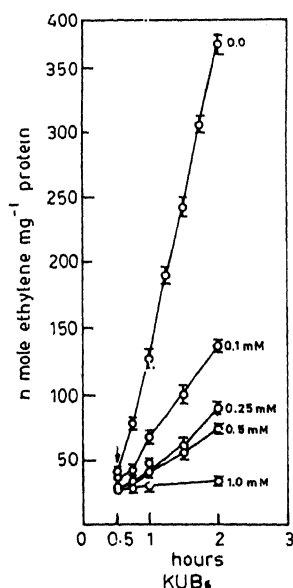
Figure 1. Ammonium repression of nitrogenase activity of KUB_6.

$1-3$ mM NH_4^+; with 5 mM NH_4^+ recovery was noted at 6 h. When NH_4^+ was introduced 30 min after the onset of the nitrogenase activity, acetylene reduction was progressively inhibited with increase in NH_4^+ concentration during the first 2 h. The inhibitory effect of NH_4^+ was almost immediate. While the cells recovered from the inhibitory effect of low concentrations of NH_4^+ ions, no recovery was noted when NH_4^+ concentration was raised to 1 mM (figure 1).

2,4-Dinitrophenol, sodium azide and *p*-chlormercuribenzoate completely inhibited nitrogenase activity at $2·5 \times 10^{-4}$ M.

The nitrogenase activity of 4 cultures of *C. tropicalis* KUB_1, KUB_6, KUC_{45} and KUG_6 were all sensitive to O_2. When both O_2 and C_2H_2 were introduced simultaneously at the beginning, the inhibition increased with increasing concentrations of O_2 (figure 2). Although the cells slowly recovered from the inhibition, the extent of recovery was less marked at higher O_2 concentrations. With 15–20% O_2 the inhibition after 22 h was 80–90%. In KUB_1 and KUC_{45} some promotion of nitrogenase activity was noted with 2% O_2; in KUB_1 the lag period was reduced to 2 h. In KUC_{45} the promotive effect was observed for the first 9 h. However, no stimulatory effect of low O_2 concentration could be detected when O_2 was introduced after commencement of nitrogenase activity (table 4). At the normal O_2 concentration of the atmosphere, nitrogenase activity was only a small fraction of that observed under anaerobic condition.

The yeast cultures possessed very little reversible hydrogenase activity. Under anaerobic condition in an argon atmosphere, no H_2 could be detected. However, small amounts of H_2 (about 50 nmol g^{-1} cell dry wt. in 48 h) could be detected under aerobic condition.

Introduction of H_2 into the vials stimulated nitrogenase activity of KUB_6, KUC_{45} and KUG_6. The optimum concentration of H_2 which promoted acetylene
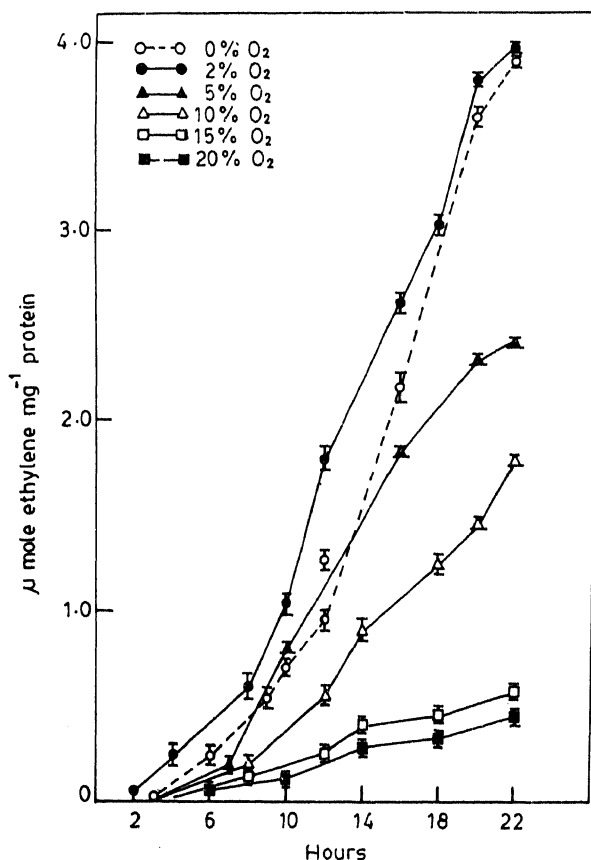
Figure 2. Oxygen sensitivity of the nitrogenase activity of the yeast isolate KUB_1.

Table 4. Effect of different concentrations O_2 on the nitrogenase activity (acetylene reduction) of the yeast isolates.

Laboratory Index No.	Rate of production of ethylene (nmol mg^{-1} protein $h^{-1} \pm SD$)				
	O_2 concentration (%, v/v)				
	0	2	5	10	20
KUB_1	452 ± 9.06	146.0 ± 2.08	253.30 ± 2.33	65.6 ± 1.73	53.33 ± 0.88
KUB_6	1172.10 ± 4.16	148.0 ± 2.30	82.33 ± 1.45	55.66 ± 2.33	25.00 ± 2.83
KUC_{45}	238.66 ± 4.09	150.0 ± 5.77	85.33 ± 2.33	48.33 ± 4.40	15.33 ± 2.66
KUG_6	1054.33 ± 33.01	176.0 ± 5.77	86.66 ± 3.33	58.33 ± 4.40	13.33 ± 3.33
CD at $P = 0.01$	104.47	26.75	13.09	24.04	18.52
CD at $P = 0.05$	63.13	16.17	7.91	14.52	11.96

O_2 was introduced into the vials just after commencement of acetylene reduction.
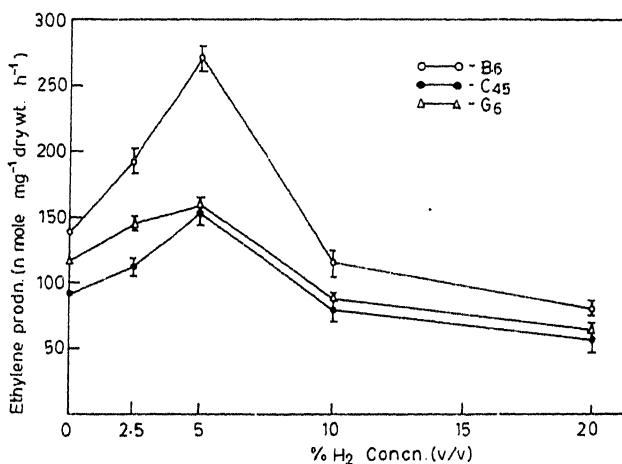
Figure 3. Effect of different concentrations of H_2 on acetylene reduction by 3 yeast isolates KUB_6, KUC_{45} and KUG_6.

reduction was 5% during a period of 24 h; higher concentrations were inhibitory (figure 3). Direct evidence of uptake hydrogenase activity was provided by an experiment in which KUB_6 and KUC_{45} were incubated with different concentrations of H_2 for 48 h. It is evident from figure 4 that both the strains actively took up H_2, the uptake increasing with concentration and with time. KUB_6 was more active than KUC_{45}.

Methane inhibited the nitrogenase activity of the yeast isolates as in the case of other diazotrophs. Although the strain KUB_6 actively reduces acetylene in the absence of methane, in the presence of methane no acetylene was reduced even after 24 h. Unlike other diazotrophs (Postgate 1982), however, even small amounts of methanol like 0.01 ml g^{-1} wet weight of cells inhibited acetylene reduction by about 90% and at higher concentrations no nitrogenase activity could be detected.
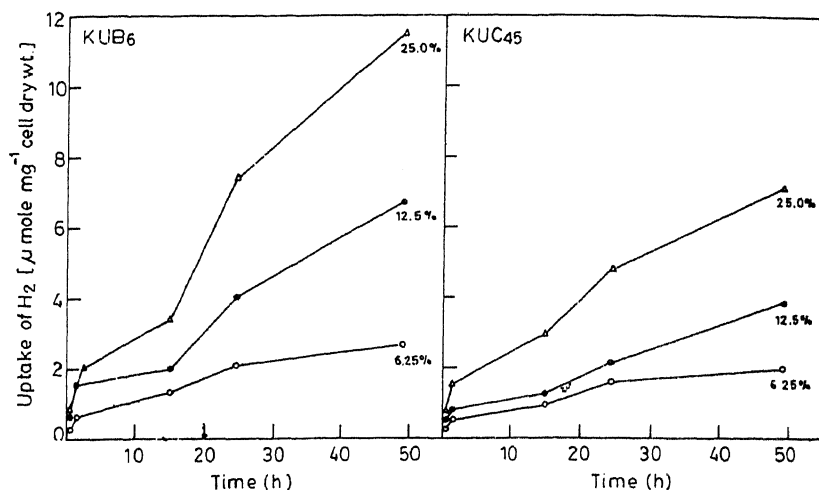
Figure 4. Uptake hydrogenase activity of the yeast isolates KUB_6 and KUC_{45}.

4. Discussion

Our observations concerning the temperature and pH optima and inhibitors of nitrogenase indicate that yeast nitrogenase has considerable similarities in its properties with those reported for nitrogenases of other diazotrophs. The optimum pH of 7·2 is within the range of pH variations of the dung samples from which they were collected, 7·18–7·38. The optimum temperature for nitrogenase was about 30°C and no nitrogenase activity could be detected at 40°C. Diazotrophy is a temperature-sensitive process (Henneckae and Shanmugham 1979) and true thermophilic diazotrophs are rare (Postage 1982). *Klebsiella pneumoniae* cannot fix N_2 at 37°C. The complete abolition of nitrogenase activity at low concentrations of 2,4-dinitrophenol, sodium azide and p-chlormercuribenzoate indicate the requirement of ATP and involvement of SH- and heavy metal enzymes in the N_2 fixation process. Sodium azide is known to inhibit the reduction of N_2 as it is itself reduced to NH_3; inhibition of ATP dependent H_2 evolution by azide is also possible.

The nitrogenase of the yeast isolates is inhibited by NH_4^+ as in other diazotrophs. As compared to other systems, however, the concentration of NH_4^+ required for complete inhibition is much higher. The strains of yeasts used also differ in their sensitivity to NH_4^+. Although nitrogenase activity is inhibited, growth is supported by NH_4^+ indicating its utilization for assimilative purposes. The yeast isolates exhibit high O_2 sensitivity and as compared to the anaerobic condition the activity became one-tenth or less when exposed to air. Scavanging of O_2 by aerobes or microaerobes in the immediate vicinity of yeast cells in the dung would decrease the O_2 to very low values. Since these diazotrophic yeast isolates are also capable of oxidizing methane to CO_2 such activities would help further in the removal of O_2. Dalton (1980) observed that diazotrophic methanotrophs are less sensitive to O_2 when metabolizing methane than when metabolizing methanol. However, the yeast isolates used could not utilize methanol either as an oxidisable substrate or for growth.

The yeast isolates like most other N_2-fixers seem to possess reversible hydrogenase and also an irreversible uptake hydrogenase. However, the reversible hydrogenase activity was very low, presumably because the H_2 evolved was rapidly taken up by the uptake hydrogenase, which seems to be quite active in this system where no H_2 evolution could be detected in an argon atmosphere. Surprisingly, H_2 evolution was detected in air, though in small amounts; the reason for this is not known at present. Although acetylene inhibits uptake hydrogenase, H_2 was consumed by the cells even in the presence of acetylene, indicating that the uptake hydrogenase activity was probably quite strong in the yeast isolates. Since H_2 also inhibits nitrogenase activity, rapid removal of H_2 by uptake hydrogenase would allow the nitrogenase to function better. Availability of H_2 in the rumen and in other anaerobic conditions due to the reversible hydrogenase of other anaerobic microorganisms associated with the yeast cells in the dung, would support uptake hydrogenase and in turn also nitrogenase activity of the cells. The uptake hydrogenase may, however, compete with the methanogens for H_2.

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Application of algal assay for defining nutrient limitation in two streams at Shillong

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Abstract. The streams selected for the study pass through deforested catchments, and
even their riparian zones are devoid of trees. Due to low level of nutrients, the streams
support reduced algal flora (14 genera; 35 species) and low epilithic algal biomass (0·07–
1·14 mg chlorophyll a m^{-2}). Persistence of high N:P atomic ratio at all sites suggests
phosphorus limiting condition. Algal assay was used to determine the effects of nitrogen,
phosphorus and trace element supplementation on algal growth potential of stream water.
Addition of phosphorus significantly increased the cell yield of test alga, *Selenastrum
capricornutum*, thereby confirming phosphorus limitation of algal growth in the selected
streams.

Keywords. Epilithic algae; diatoms; phosphorus limitation; algal assay; *Selenastrum
capricornutum*.

1. Introduction

Of some 15 elements needed for growth and metabolism of algae, the supply of
nitrogen and phosphorus is frequently limiting in natural waters. Higher
concentrations of nitrogen and phosphorus, however, accelerate algal growth and
cause serious entrophication problems in waterbodies. Phosphorus limitation of
algal growth generally occurs in lakes, whereas nitrogen limitation is common in
seas. Similar informations about lotic systems are scarce, although limited efforts
on the temperate streams of north America showed phosphorus or nitrogen limiting
conditions (Stockner and Shortreed 1978; Elwood *et al* 1981; Grimm *et al* 1981). So
far, only one report has come from the tropical region showing phosphorus
limitation of algal growth in a Costa Rican stream (Pringle *et al* 1986).
Micronutrient deficient conditions have also been reported from laboratory and
natural streams (Wuhrmann and Eichenberger 1975; Pringle *et al* 1986).

The study was initiated with the premise that literature on the ecology of stream
algae is almost non-existent in India. This paper identifies nutrient limitation of
algal growth in two deforested streams. Bioassays were performed with the test alga
Selenastrum capricornutum due to its sensitive reaction to nutrients and toxicants
(US EPA 1971).

2. Study area

Two streams, Wah Umkhen (fourth order) and Unnamed (third order), draining
deforested catchments, were selected in Shillong (Meghalaya, India). Table 1 gives
important physiographic features of the selected streams. The two stations of Wah

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Table 1. Important features of the selected streams.

Parameter	Station	Wah Umkhen	Unnamed
Altitude (m)	1	1696	1583
	2	1587	
Latitude	1	25°33′ N	25°33′ N
	2	25°32′ N	
Longitude	1	91°57′ E	91°54′ E
	2	91°54′ E	
Width (m)	1	1·3–8·3	0·40–3·20
	2	3·1–11·0	
Depth (cm)	1	13·0–70·0	4·0–22·0
	2	8·0–80·0	

Umkhen are located approximately 1 km apart. Only one station of the Unnamed stream has been considered for sampling. The stream bed consists of granite and quartzite rocks ranging from gravel to boulder, interspersed with silty to sandy sediment. All sites are totally illuminated due to the absence of trees in the riparian zone. On cloudless days, PAR at the stream surface was approximately 1500 μ mol m^{-2} s^{-1} at 12 noon.

3. Materials and methods

Sampling was carried out in October 1988, March 1989 and June 1989, respectively falling under winter, summer and rainy seasons. At all stations, 3 l stream water was collected in polyethylene bottles for various analyses. Flow rate was measured using a float, pH with an Ingold combination electrode, and conductivity with a Systronics conductivity meter (model 304). Ammonia-nitrogen and NO_3–N were analyzed colorimetrically by phenol hypochlorite and brucine-sulfanilic acid methods, respectively. Soluble reactive phosphorus (PO_4–P) was estimated by ascorbic acid method, and total phosphorus by the same method after digestion with 4% ammonium persulphate. Silicate was estimated by molybdosilicate method. All analyses were according to Wetzel and Likens (1979). Except flow rate (n = 9), all measurements were carried out in triplicate, Calcium and magnesium were estimated by a Perkin Elmer atomic absorption spectrophotometer (model 2380) after adjusting the pH of the samples to <2.

Epilithic algae were scraped from 4·5 cm^2 area of rocks, and the epipelon were aspirated randomly from the surface sediment with a pipette. The samples were diluted with 5 ml water and preserved in 5% formaldehyde solution for algal identification and enumeration. One half of the aliquot was kept aside for identification of algae other than diatoms. The other half was boiled with concentrated nitric acid for the identification of diatoms. Three hundred diatom valves were counted in random fields under 1000 × magnification of Carl Zeiss microscope (model laboval 4) using a Spencer's brightline hemocytometer.

Algal assay procedure-bottle test was used to determine nutrient limiting condition (US EPA 1971). The test alga *S. capricornutum* was obtained from Dr Olav M Skulberg, Norwegian Institute of Water Research, Oslo. The synthetic

algal nutrient medium (US EPA 1971) was used for the maintenance of stock cultures at $24 \pm 1°C$ in a 14 h light (PAR 70 μmol m^{-2} s^{-1}) and 10 h dark cycle. The test waters were filtered, autoclaved and aseptically spiked with various concentrations of nitrogen (NO$_3$–N), phosphorus (PO$_4$–P) and trace elements in different combinations (see table 2). Experiments were carried out in 38×150 mm culture tubes containing 10 ml of test waters with or without various spikes. *S. capricornutum* was inoculated in each culture tube at an initial density of 10^{-3} cells ml^{-1}. The culture tubes were incubated under conditions used for maintaining the stock cultures. The tubes were hand-shaken twice daily to resuspend the cells. The maximum standing crop (algal cell count on the 14th day) was measured with the help of a hemocytometer.

Cell count data were log-transformed and subjected to ANOVA. Least significant difference ($P < 0.05$) was calculated to compare the algal biomass for various treatments (Snedecor and Cochran 1967).

4. Results

Table 3 depicts important physico-chemical characteristics of stream water at different stations. Velocity, conductivity, and the level of nutrients exhibited considerable variation. Marked fluctuations in velocity were observed, with lowest value in March (10–33 cm s^{-1}) and highest in June (62–112 cm s^{-1}). The pH was much more closely confined over space and time, but conductivity showed an inverse relation with flow. Ammonia level was highest during June (56–73 μg l^{-1}) and lowest in October (10–17 μg l^{-1}). Nitrate-nitrogen concentration was maximum in October. Soluble-reactive phosphorus and total phosphorus showed peak in October. Changes in dissolved silica were not pronounced, except at station 1 of Wah Umkhen. The concentrations of calcium and magnesium decreased tremendously in October at all stations.

The number of taxa representing the epilithon and the epipelon was highest in Unnamed stream, except during October when station 2 of Wah Umkhen showed the highest. During June, minimum number of taxa occurred in the epipelon at

Table 2. Experimental design for algal bioassay.

No.	Treatment
1.	Stream water (control)
2.	Stream water + 1.0 mg l^{-1} N
3.	Stream water + 0.5 mg l^{-1} N
4.	Stream water + 0.05 mg l^{-1} P
5.	Stream water + 0.025 mg l^{-1} P
6.	Stream water + 0.5 mg l^{-1} N + 0.05 mg l^{-1} P
7.	Stream water + 1.0 mg l^{-1} N + 0.05 mg l^{-1} P
8.	Stream water + 0.5 mg l^{-1} N + 0.025 mg l^{-1} P
9.	Stream water + trace elements
10.	Stream water + trace elements + 0.5 mg l^{-1} N
11.	Stream water + trace elements + 0.05 mg l^{-1} P
12.	Stream water + trace elements + 0.5 mg l^{-1} N + 0.05 mg l^{-1} P
13.	Full strength medium

Table 3. Physico-chemical attributes of stream water at different stations.

| | October 1988 | | | March 1989 | | | June 1989 | | |
| | Wah Umkhen | | | Wah Umkhen | | | Wah Umkhen | | |
Parameter	St. 1	St. 2	Unnamed	St. 1	St. 2	Unnamed	St. 1	St. 2	Unnamed
Flow rate (cm s^{-1})	54·8	32·4	28·3	39·8	32·3	10·4	112·4	78·5	62·6
pH	6·8	6·9	6·0	6·4	6·4	5·6	6·4	6·9	5·7
Conductivity (μS cm^{-1})	353	447	180	373	553	473	360	393	207
Nitrate-nitrogen (mg l^{-1})	2·73	2·53	1·75	0·99	0·90	2·11	0·65	0·81	0·26
Ammonia-nitrogen (μg l^{-1})	17	10	10	44	80	ND*	56	73	69
Soluble reactive phosphorus (μg l^{-1})	9·0	7·3	1·0	1·3	0·8	9·0	0·6	1·7	0·3
Total phosphorus (μg l^{-1})	18·0	20·0	16·0	2·1	3·9	17·5	5·6	7·9	5·8
Dissolved silica (mg l^{-1})	8·0	12·5	11·2	7·0	10·4	10·0	11·3	11·6	7·5
Calcium (μg l^{-1})	125	152	81	348	171	382	144	361	174
Magnesium (μg l^{-1})	113	84	36	415	228	219	241	442	386

*Concentration below the limit of detection.

both the stations of Wah Umkhen, and high flow rate made epilithic flora difficult to study. Maximum number of species was encountered during March and dominant species, most of which are members of Bacillariophyta, are listed in table 4.

Figures 1 to 3 show that the maximum standing crop of test alga was obtained by phosphorus addition to the test waters. Phosphorus in combination with nitrogen yielded better results. Phosphorus supplementation to test waters yielded higher biomass at 0·05 mg l^{-1} than at 0·025 mg l^{-1}, except at station 1 of Wah Umkhen in October and at the Unnamed stream in March. Amongst the various treatments, enrichment of stream water with 10 mg l^{-1} nitrogen and 0·05 mg l^{-1} phosphorus increased the standing crop maximally. Nitrate and trace element addition did not elicit marked effect. None of the treatments levelled the results shown by the full strength medium.

5. Discussion

The low level of nitrogen, phosphorus, calcium and magnesium suggests that the streams are oligotrophic and softwater by nature. Ammonia-nitrogen was at an extremely low level because the streams are thoroughly aerated without excessive organic loading. The high N : P atomic ratio suggests the streams to be phosphorus deficient. The bioassay results supported this contention because algal growth was increased by phosphorus supplementation to the stream water. Phosphorus-limiting conditions have been reported from some temperate streams also (Peterson *et al* 1983; Pringle and Bowers 1984).

Table 4. Important structural features of algal communities in Wah Umkhen and Unnamed streams.

| Date | Parameter | Wah Umkhen | | Unnamed |
		St. 1	St. 2	
October 1988	Epilithic biomass (Chlorophyll *a*, mg m^{-2})	$2 \cdot 1 \pm 0 \cdot 10$*	$1 \cdot 5 \pm 0 \cdot 06$	$1 \cdot 7 \pm 0 \cdot 04$
	Total number of species (genera)	14 (8)	17 (10)	16 (7)
	Major species	*Synedra ulna* (Nitz.) Ehr., *Navicula subtenelloides* Cholnoky and *Caloneis ventricosa* (Ehr.) Meist.	*S. ulna*, *N. subtenelloides* and *Navicula cryptocephala* Kütz.	*C. ventricosa*, *Gomphonema parvulum* (Kütz.) Grun., *N. subtenelloides N. cryptocephala* and *Navicula radiosa* Kütz.
March 1989	Epilithic biomass (chlorophyll *a*, mg m^{-2})	$2 \cdot 8 \pm 0 \cdot 05$	$2 \cdot 1 \pm 0 \cdot 04$	$11 \cdot 4 \pm 0 \cdot 46$
	Total number of species (genera)	15 (8)	15 (9)	19 (11)
	Major species	*N. cryptocephala S. ulna* and *Eunotia pectinalis* (Kütz.) Rabh.	*N. cryptocephala*, *N. subtenelloides S. ulna* and *C. ventricosa*	*N. cryptocephala*, *N. subtenelloides* and *C. ventricosa*
June 1989	Epilithic biomass (chlorophyll *a* mg m^{-2})	—	—	$0 \cdot 07 \pm 0 \cdot 07$
	Total number of species (genera)	8 (4)**	7 (4)**	12 (6)
	Major species	*N. cryptocephala*, *N. subtenelloides*, *S. ulna* and *Eunotia pseudoparallela* A°Berg	*N. cryptocephala*, *S. ulna*, *N. subtenelloides* and *Pinnularia braunii* (Grun.) Cleve	*N. cryptocephala*, *S. ulna*, *Gomphonema gracile* Ehr. and *N. subtenelloides*

*Mean $\pm$ SEM (n = 3).
**Includes only epipelic taxa, as high flow rate did not allow the sampling of epilithic. communities.

The total number of species encountered during the study is much less than the previous reports (O'Quinn and Sullivan 1983; Rushforth and Squires 1986). Depauperate algal flora of streams has been ascribed to nutrient deficiency (Chessman 1986) or low pH (Keithan *et al* 1988). Phosphorus-limiting condition seem to be responsible for the diminutive algal flora in the present study. Furthermore, in the present work low pH does not seem to have exerted a major influence because many of the taxa encountered by us have been previously reported at pH 7, with best development above 7 (Lowe 1974).

Biomass peak during summer ($2 \cdot 1$–$11 \cdot 4$ mg m^{-2}) was much lower than in a softwater stream studied by Marker (1976). He obtained biomass values ranging
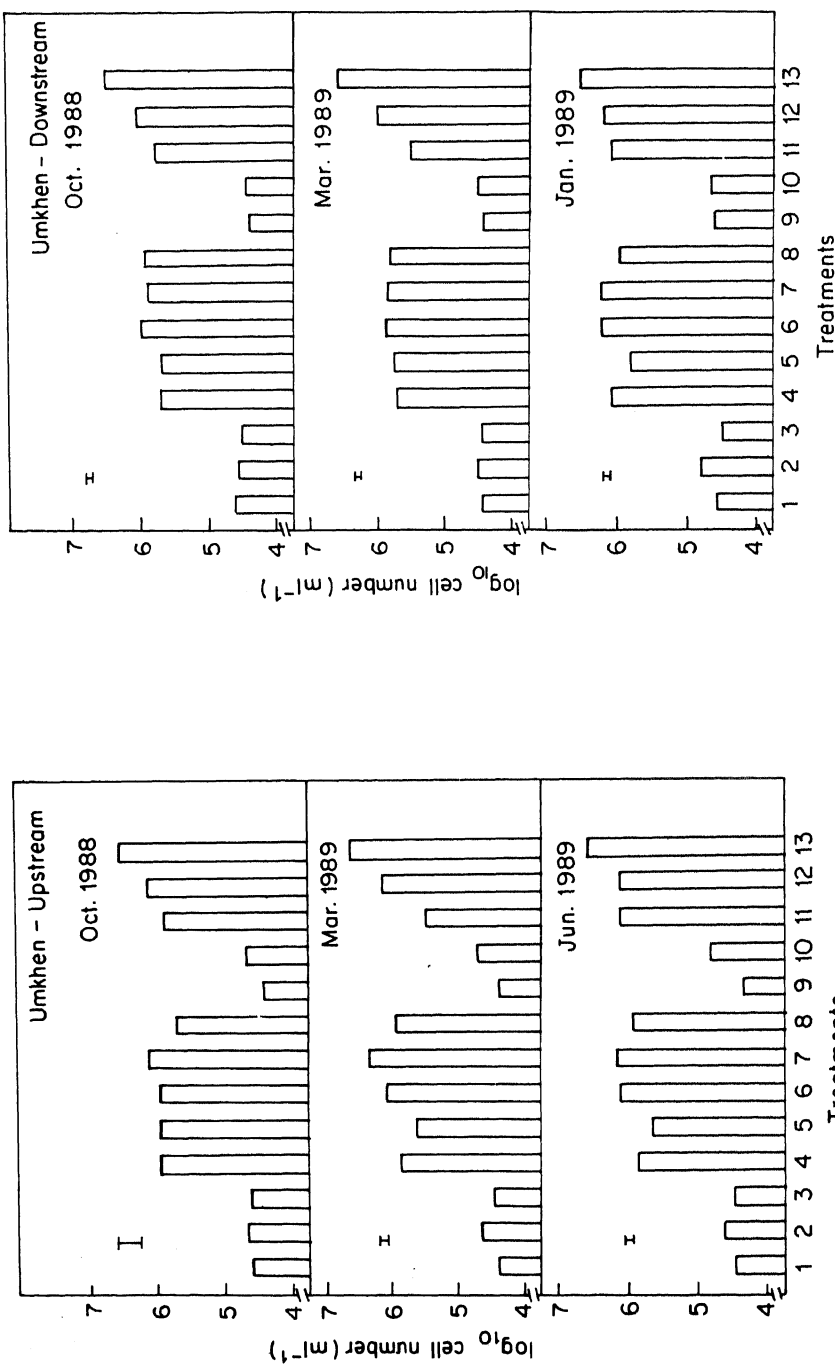
Figure 1. Wah Umkhen-upstream station. Cell yield of *S. capricornutum* on the 14th day in test water supplemented with N, P and trace elements. Treatments have been explained in table 2. Vertical bars show LSD ($P < 0.05$).

Figure 2. Wah Umkhen-downstream station. Final yield of *S. capricornutum* on the 14th day in test water enriched with N, P and trace elements. Treatments as in table 2. Vertical bars show LSD ($P < 0.05$).
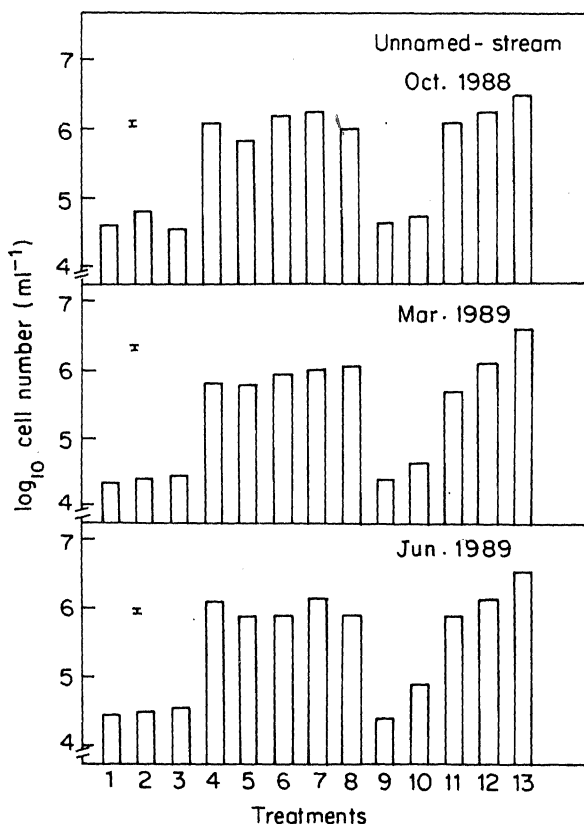
Figure 3. Unnamed station. Maximum standing crop (14th day) of *S. capricornutum* in test water with N, P and trace element supplementation. Treatments as in table 2. Vertical bars show LSD ($P < 0.05$).

from below $10 \, \text{mg m}^{-2}$ in winter to $50 \, \text{mg m}^{-2}$ in summer. Year-long domination of the epilithic community by diatoms may explain the low chlorophyll *a* accrual in the selected streams (see La Perriere *et al* 1989).

Soluble reactive phosphorus saturates the growth of algae at various levels: $>7 \, \mu\text{g l}^{-1}$ in filamentous algae (Seeley 1986), and $<4 \, \mu\text{g l}^{-1}$ in case of diatoms (Bothwell 1985). The range of SRP in our case was far lower ($0.3–1.7 \, \mu\text{g l}^{-1}$), except at stations 1 and 2 of Wah Umkhen in October and station 1 of the Unnamed stream in March. Jones *et al* (1984) demonstrated higher chlorophyll *a* for Missouri streams with low phosphorus concentrations, whereas Krewer and Holm (1982) reported positive relation between chlorophyll *a* and total dissolved phosphorus in artificial streams.

Increased biomass with phosphorus addition in bioassay experiments suggests that phosphorus deficiency is limiting algal productivity in the selected streams. As a consequence, the standing crop of stream algae is yet to reach the nuisance level (100–150 mg chlorophyll *a* m^{-2}, Welch *et al* 1988). It may however be attained if the level of nutrients, particularly phosphorus, in these streams is further increased due to intensification of disturbances in the catchments.

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Modes of entry, establishment and seed transmission of *Peronospora parasitica* in radish

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Abstract. The entry of *Peronospora parasitica* conidia through stigma, ovary wall and its establishment in the ovary is clearly demonstrated. The pathogen also enters directly through the inflorescence axis of the mother plant. The infection through stigma and ovary wall results in embryonal infection. The infected seeds transmit the downy mildew disease and a direct correlation is noticed between embryo infection and seed transmission of the pathogen.

Keywords. *Peronospora parasitica*; artificial inoculation; seed transmission; *Raphanus sativus*.

1. Introduction

Peronospora parasitica (Pers. ex Fr.) Fr. causing downy mildew in *Raphanus sativus* L. is found to be seedborne (Jang 1989). Conidial penetration among the Crucifers has been observed in many parts of the plants other than the seeds (Chu 1935; Chou 1970). However the modes of entry, establishment and transmission of the pathogen in the seeds of radish have not been reported. They are investigated in the present study.

In other downy mildews, the seedborne nature and mode of transmission have been well documented (Safeeulla and Shetty 1974; Shetty *et al* 1979; Prabhu *et al* 1983).

2. Materials and methods

2.1 *Infection through stigma*

To study the entry of conidia through stigma, unfertilised stigma of healthy plants were taken from 4 cultivars viz., Japanese white, Arka nishant, Pusa desi and Pusa reshmi, and sown in the field at Downy Mildew Research Laboratory (DMRL). The inoculum was prepared following the technique of Safeeulla (1976). The conidia, after incubation of infected leaves at 16°C, overnight, were scraped off and a conidial suspension containing about 20,000–30,000 conidia/ml was prepared. Artificial inoculation was done by two methods:
(i) Unfertilised carpels were removed from healthy plants. The ovaries along with style and stigma were placed on sporulating surface of infected leaves at 16°C, for 3 days. At 12 h intervals such ovaries were fixed in acetic acid alcohol (1:3) and subjected to alkali maceration technique (Shetty *et al* 1978).

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(ii) Unpollinated carpels were sprayed with conidial suspension at 0100 h. Inflorescences of healthy plants were dipped in a container with a concentrated conidial suspension. Such treated carpels were covered with moist polyethylene bags to maintain humidity for 2–3 days. The carpels were then fixed in acetic acid alcohol. They were dehydrated by boiling in alcoholic lactophenol (50:50) for 30–35 min followed by maceration in 5% KOH solution for 24 h. The macerated carpels were washed in distilled water and treated with saturated chloral hydrate solution with 0.5% cotton blue for 24 h. The clear ovaries were mounted in lactophenol on slides after squashing and observed microscopically.

For the study of the most susceptible stages of infection sprayings were done at pre-stigma, stigma and post-stigma emergence stages.

Further, to study the percentage of infection, the seeds from previously sprayed inflorescences were subjected to alkali maceration.

2.2 Infection through ovary wall

Unpollinated carpels from healthy plants were selected. The stigma and style were exised leaving only the ovary and the cut ends were plugged with wax. The downy mildew infected leaves were kept for sporulation following the same technique (Safeeulla 1976). The exised ovaries were subjected to dehydration in alcoholic lactophenol (50:50) by boiling for 30–35 min, followed by maceration with 0·5% cotton blue stain for 24 h. Samples were collected after 12, 24, 48 and 72 h. The processed ovaries were squashed and observed under compound microscope.

2.3 Infection through mother plant

Seeds were surface sterilised using 0.1% mercuric chloride for 5 min followed by 5 washings in sterile distilled water. Such treated seeds were sown in pots containing steam sterilised soil (20 pound pressure for 15 min) and kept in glass house which was free from air-borne inoculum. Flower buds from these plants were collected and ovaries from such plants were macerated using alkali maceration technique.

2.4 Transmission of mycelium through seeds

The seeds were collected from the inflorescences previously sprayed with the conidial suspension, dried under natural conditions and stored at laboratory temperature (24–26°C). The percentage of seeds with mycelium was found out using the maceration technique with 400 seeds for each sample. In another set, 400 seeds were sown, from the above sample under controlled conditions in glass house which was free from air-borne inoculum. Before sowing the seeds were treated as described (sect. 2·3). After seedling emergence, observation was made daily and disease incidence was recorded. The infected seedlings were plucked off as soon as symptoms appeared so as to prevent the spread by air-borne spores. The same experiment was repeated in DMRL field.

3. Results

3.1 *Infection through stigma*

The entry of the pathogen into the carpel was observed through stigma, ovary wall, and systemically infected parents plants. Under *in vitro* and *in vivo* conditions, artificial inoculation resulted in the entry of conidia into stigma. After 24 h, conidia were found germinating and penetrating the stigmatic lobes (figure 1a). After 48 h, the stylar canal was found to be colonised by the mycelium (figure 1b). After 2–3 days of inoculation, mycelium was seen invading the ovary (figure 1c), forming a network of branched coenocytic mycelium. Of the three stages, the stigma emergence was highly susceptible.

3.2 *Infection through ovary wall*

Entry of conidial germ tube through ovary wall (figure 1d) and its establishment in the unfertilised ovary were observed within 24 h of infection (figure 1e).

3.3 *Infection through mother plant*

Macerated ovaries from systemically infected plants showed the presence of mycelium in the ovary wall (figure 1f).

3.4 *Transmission of mycelium through seeds*

The seeds collected from previously sprayed inflorescences showed the presence of mycelium in the embryo. The percentage of embryonal infection varied with cultivars (table 1).

4. Discussion

The carpel infection through, ovary wall and mother plant has been well demonstrated in the present investigation. The carpel infection through stigma has been carried out in several fungal pathogens (Neergaard 1977). In downy mildew of pearl millet, the establishment of mycelium in the seeds through the stigmatic infection by zoospores was confirmed (Subrammanya *et al* 1981).

Stigma emergence stage has been found to be very susceptible for conidial infection of *P. parasitica* in the present study. This observation is of great importance in understanding the epidemiology of the downy mildew disease in radish. Failure of infection of the fertilised ovary may be attributed to the development of certain morphological and physiological changes in the ovary wall after pollination in pearl millet (Subrammanya *et al* 1981). Neergaard (1977) has emphasised that the penetration of many fungi into deeper layers of seeds is prevented after pollination by development of such barriers.

The significant observation in the present study is the entry of pathogen into the embryo. The percentage of seed transmission is in direct correlation with that of
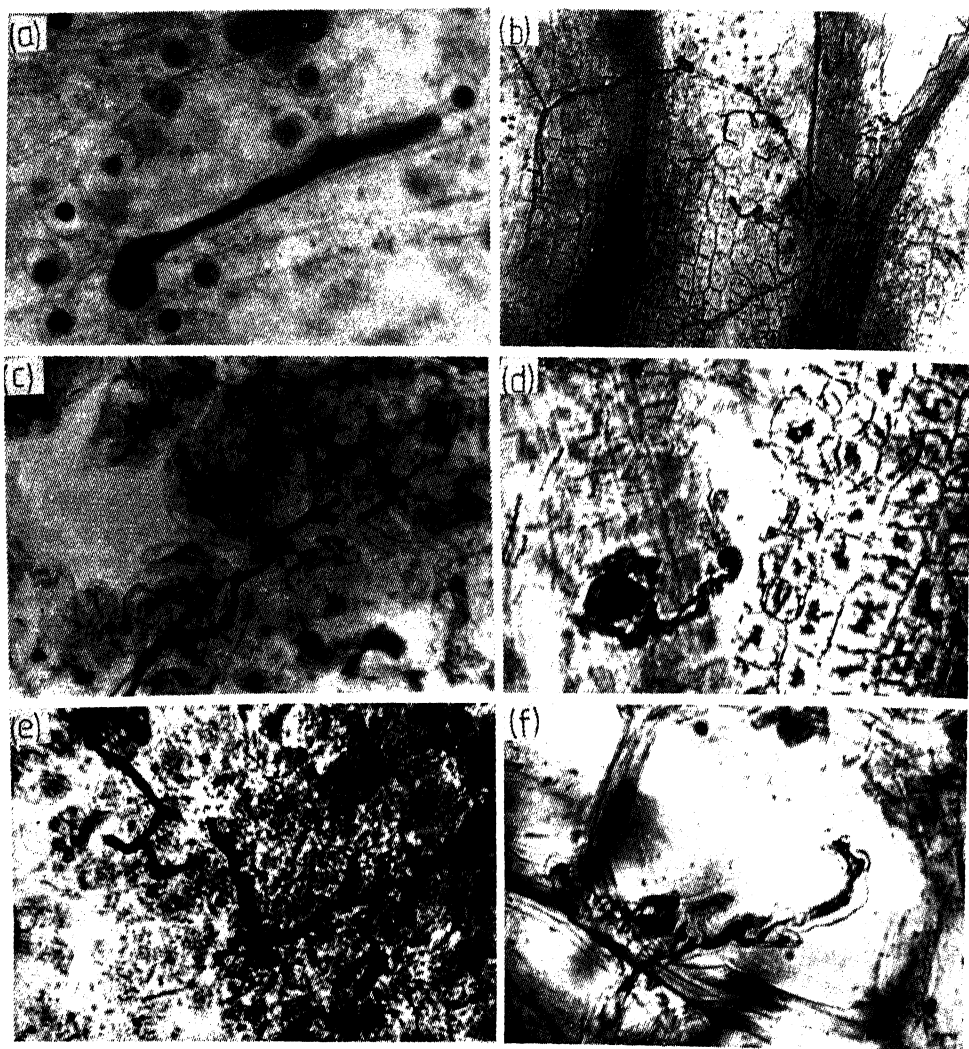
Figure 1. Artificial inoculation of *P. parasitica* in *R. sativus*. (**a**) Conidial entry through the stigma after 24 h (× 800). (**b**) Mycelium invades stylar tissues after 48 h (× 100). (**c**) Invasion of ovary after 2–3 days (× 200). (**d**) Entry of conidial germ tube through ovary wall (× 500). (**e**) Establishment of mycelium in ovary through ovary wall within 24 h of inoculation (× 600). (**f**) Mycelium in ovary wall of systemically infected plants (× 100).

embryonal infection. This is in accordance with the observation made in pearl millet (Shetty and safeeulla 1980). Survival chances of obligate parasite in living embryonal tissue are more and hence such a correlation is significant. Even if the percentage of embryonal infection is small as seen in the cultivar, Pusa reshmi, this amount is sufficient to take heavy toll on the crop yield.

Exchange of seeds is common and the danger of introducing new pathogenic races with the germ plasm into new areas is becoming increasingly clear (Shetty and

Table 1. Percentage of seeds showing embryonal infection after artificial inoculation of *P. parasitica* in *R. sativus*.

	Seeds showing infection in		
Cultivar	Laboratory[a]	Glass house[b]	Field[c]
Japanese white	20	15·0	22·5
Arka nishant	10	11·0	13·0
Pusa desi	5	6·0	10·0
Pusa reshmi	2	1·5	3·0

[a] Seeds collected from previously sprayed inflorescence.
[b,c] Sample from '*a*' sown in glass house and field.

Safeeulla 1980). Hence, it is necessary to examine the seed lot for downy mildew inoculum. This could be applicable for other crucifers too.

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Laboratory experiments on competition between two rhizoplane microfungi *Penicillium chrysogenum* and *Trichoderma harzianum*

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Abstract. A field study of rhizoplane microfungi associated with little bluestem (*Schizachyrium scoparium* (Michx.) Nash), a common tallgrass prairie species, had indicated that *Penicillium chrysogenum* Thom and *Trichoderma harzianum* Rifai outcompeted each other in unfumigated and fumigated soils, respectively. To investigate the mechanisms of competition, interactions between the two fungi were studied in laboratory experiments using an artificial model system. Mutual suppression did occur. The first-inoculated fungus was less affected. Some detrimental residual effects on the growth of one species were observed when it was grown along with the killed mass of the other in the same medium.

Keywords. Competition; methyl bromide; microfungi; rhizoplane; *Penicillium*; *Trichoderma*.

1. Introduction

A number of mechanisms act in individual and concerted ways to shape the rhizosphere composition (Fitter 1985). Competition among the root-surface microfungi is one of them. Laboratory experiments on fungal competition are numerous (e.g., Tribe 1966; Armstrong 1976), but few studies have examined interactions between members of the root-surface epiphytic mycoflora in laboratory conditions. Because these fungi utilize root exudates (Rovira 1969) and are directly influenced by the roots (Cook and Snyder 1965), they are expected to show exploitative competition for space and nutrients. Other kinds of interactions (e.g., interference competition) may also influence these fungi (Brian 1960). Further, production of toxins by some have inhibitory effects on other co-habiting microorganisms (Czachor 1986a, b).

In preliminary experiments comparing the rhizoplane mycoflora associated with little bluestem (*Schizachyrium scoparium* (Michx.) Nash), a common tallgrass prairie species, *Penicillium chrysogenum* Thom and *Trichoderma harzianum* Rifai were the dominant species in the unfumigated (frequency value 18%) and fumigated (frequency value 36%) soils (fumigation with methyl bromide), respectively ($P < 0.05$, Bandyopadhyay 1987). In the present study, presence of interspecific competition between these two fungi and mechanisms behind such competition were investigated under laboratory conditions.

2. Materials and methods

All experiments were carried out at the same time and under the same general conditions. Malt extract was used as the liquid test medium because it was suitable to both *P. chrysogenum* and *T. harzianum*. Fifty ml of 2% medium (pH 6) were distributed in each 250 ml Erlenmeyer flask; the flasks were cotton-plugged and

sterilized. For all treatments, a 1 cm square of agar cut from a 7-day-old suspension-seeded 2% malt agar plate served as inoculum for each flask, and was floated carefully, agarside down, on one side of the liquid medium. When two different organisms were inoculated in the same flask, they were placed on opposite sides (previously labelled on the flask) of the medium. Three replicate flasks for each harvest day were prepared for each treatment. Care was taken to ensure that the inocula stayed in the same spot throughout the experiment. Because *T. harzianum* had deep green conidia (Rifai 1969), while conidia of *P. chrysogenum* were greyish turquoise (Pitt 1985), it was possible to differentiate them throughout the experiment. Inoculated flasks were incubated at 21°C. Mycelial growth of the two different organisms usually remained separate. In few cases where they did make contact, the mycelia did not grow over one another or become intercalated. Thus, it was always possible to separate them carefully at the time of harvesting. The mycelial mat from each replicate flask was separately harvested for each harvest day. After each harvest, the mycelial mat was dried at 80°C for 12 h and weighed.

The individual experiments were designed as follows (T and **P** stand for *T. harzianum* and *P. chrysogenum*, in a given treatment, respectively. Also, superscripts "t" and "p", used for the biomass, refer to *T. harzianum* and *P. chrysogenum*, respectively):

Experiment 1:   In order to test for competitive interaction, cultures of **T, P** and **T** and **P** were grown together for 10 days, and 3 flasks were harvested every 48 h, beginning on day 2 (treatments designated as **T, P,** and **TP**).

Experiment 2:   Priority effects were tested as follows: **T** was added first to the medium, **P** was added on day 2, and they were grown together for 8 more days; each one separately harvested every 48 h (treatment designated as **T + P**). Similarly, in the other set, **P** was added first; **T** was added on day 2, and grown together for 8 more days; each one separately harvested every 48 h (treatment designated as **P + T**).

Experiment 3:   Residual effects of the killed mycelium of one on growth of the other organism were tested. At first P was added. On day four, 5 ml of concentrated (99·5% pure, BDH, UK) liquid methyl bromide (**mb**) was applied by means of a small volume applicator (modified after Amstutz 1968), the liquid was vaporized by immersing the applicator reservoir in warm water (Gandy and Chanter 1976) and the vapor was passed into the flasks through a tube. The flasks were then kept plugged for 12 h, and subsequently the methyl bromide vapor was allowed to escape completely from the building over a period of another 2 h. The amount, concentration, and duration of treatment with this substance, as well as the evaporation time had been tested previously in some experiments involving the same test organisms; these experiments were modified after those by Gandy and Chanter (1976) and Ebben *et al* (1983). Identical application of **mb** before the treatments of experiments had given the same results as experiment 1. Then **T** was added in these flasks, and grown for 6 more days with a harvest every 48 h (treatment designated as **PmbT**); a similar experiment was performed with **T** added first, killed with **mb**, and subsequent addition of **P** (treatment designated as **TmbP**).

For statistical purposes in all tests, biomass of growth after 6 days was analyzed using ANOVA, followed by Tukey's mean separation procedure for pairwise comparisons.

3. Results

The dry biomasses (in mg) obtained from different experiments are given in table 1.

Biomass of *T. harzianum* reached its maximum on day 6, while that of *P. chrysogenum* reached its maximum on day 8. It was also observed that the biomass of *T. harzianum* in the combined growth of *T. harzianum* and *P. chrysogenum* was lower than that when grown alone (^{t}TP < ^{t}T). Likewise, biomass of *P. chrysogenum* in the combined growth of *T. harzianum* and *P. chrysogenum* was less than that when grown alone (^{p}TP < ^{p}P).

In the priority-effect treatments (experiment 2) the biomass of *T. harzianum* when grown first of the two organisms (^{t}T + P) was greater than ^{t}TP. Both ^{t}T + P and ^{t}TP were greater than the biomass of *T. harzianum* with priority of *P. chrysogenum* (^{t}P + T). Likewise, the biomass of *P. chrysogenum* in the combination growth when added first (^{p}P + T) was greater than that when the two were grown for the same time (^{p}TP), and both ^{p}P + T and ^{p}TP were greater than the biomass of *P. chrysogenum* when added second (^{p}T + P).

In the residual-effect treatments (experiment 3), when *T. harzianum* was inoculated first, the biomass of *P. chrysogenum* (^{p}TmbP) was lower than the biomass of *P. chrysogenum* when it was added second after *T. harzianum* in the absence of methyl bromide (^{p}T + P). In the reversed case, the biomass of *T. harzianum* (^{t}PmbT) was similar to ^{t}P + T.

4. Discussion

The experiments have shown that the two species affect each other adversely in a closed laboratory system (^{t}TP < ^{t}T and ^{p}TP < ^{p}P). It is therefore possible that these two organisms compete in nature as well. *P. chrysogenum* is at an advantage in unfumigated soil because of its easy spread, and *T. harzianum* is well known to be favored by all kinds of fumigation because of its rapid recolonization capacity.

It was expected that the first-inoculated species would be at an advantage. The results (^{t}T + P > ^{t}TP > ^{t}P + T and ^{p}P + T > ^{p}TP > ^{p}T + P) confirm this expectation. The priority effect might imply the occupation of more resource and space. In unfumigated soil, *P. chrysogenum* was probably a pre-existing species. In the fumigated soil, the same applies to *T. harzianum*.

Table 1. Mean biomass of mycelial growth in different experimental conditions.

| | Mean dry biomass (mg)[a] | | | | | | | | | |
| | Expt 1 | | | | Expt 2 | | | | Expt 3 | |
Day	^{p}P	^{t}T	^{p}TP	^{t}TP	^{p}P + T	^{t}P + T	^{p}T + P	^{t}T + P	^{p}TmbP	^{t}PmbT
2	77 ± 20	77 ± 7	34 ± 3	13 ± 6	66 ± 6	—	—	72 ± 6	—	—
4	92 ± 8	89 ± 9	38 ± 8	32 ± 9	91 ± 20	8 ± 2	12 ± 7	99 ± 2	—	—
6[b]	106 ± 40	116 ± 9	47 ± 1	56 ± 9	97 ± 2	35 ± 8	22 ± 5	117 ± 10	5 ± 11	37 ± 3
8	118 ± 34	101 ± 7	53 ± 7	52 ± 4	101 ± 1	47 ± 2	30 ± 20	108 ± 20	17 ± 2	46 ± 20
10	98 ± 50	81 ± 10	53 ± 2	31 ± 10	97 ± 20	56 ± 30	30 ± 2	93 ± 8	25 ± 8	57 ± 1

[a]Mean of 3 replicates ± SE. [b]Least significant difference on day 6 (sample day used for statistical analyses): 12·5 for T, and 14·5 for P.

It was expected that killed residues of one species might adversely affect the other organism, perhaps due to production of toxins. The results showed that ^tPmbT was equal to $^tP + T$, but ^PTmbP was distinctly lower than $^PT + P$. Thus the residual effects of *T. harzianum* were larger than those of *P. chrysogenum*. The mechanism for this effect is unknown. Depletion or alteration of the resources in the medium may be one of the reasons.

Although this study in an artificial, nutrient-rich, and closed environment can not claim to mimic real situations in the soil, it could well be used as an artificial model to highlight the parallelism between the field and laboratory systems.

Acknowledgements

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Microfungal species associated with the gut content and casts of *Drawida assamensis* Gates

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Abstract. Microfungi were isolated from earthworm (*Drawida assamensis*) gut contents and freshly laid worm casts of a pineapple plantation field using Warcup's soil plate method. A total of 17 species of microfungi were isolated, out of which 16 occurred in the anterior region, 12 in the middle region and 10 in the posterior region of the gut and 10 in the worm cast respectively. One species was restricted to posterior region of the gut and the worm cast. The digestion of microfungi in earthworm's digestive tract occurred in a trend anterior > middle > posterior.

Keywords. *Drawida assamensis* Gates; microfungi; pineapple.

1. Introduction

Selective feeding habit of earthworms and the passage of the ingested materials in worm gut influence the properties of worm faeces (Lee 1983). A number of investigators have reported that the casting and excretion of worms may indirectly improve the nutrient supply to plants (Mulongoy 1986; Krishnamoorthy and Vajranabhiah 1986; Tiwari *et al* 1989). Studies on microflora of the intestinal tract of earthworms have so far received little attention from soil microbiologists (Parle 1963; Dash *et al* 1979; Gorbenko *et al* 1986). For a better understanding of the effect of earthworm ingestion on microbial processes in soils, knowledge of the microbial biomass in digestive tract and casts of earthworm may be useful. Therefore, the present study was undertaken to determine the microfungal population of the gut of earthworm and its cast.

2. Materials and methods

The study was carried out at Pineapple Research Station, Nayabanglow (latitude 25°44'N, longitude 91°53'E, altitude 800 M) in the east Khasi hills of Meghalaya about 30 km north of Shillong. The study soil is a red sandy loam (sand 69%, silt + clay 31%) of laterite origin (oxisol). The pH of the soil was 4·96 and the organic carbon and nitrogen contents were 1·6% and 0·4% respectively. Soil temperature varied between 16°C and 26°C.

In the present investigation the culturing of microfungi of gut content and the worm cast have been used to investigate the fungal communities of the gut and cast of earthworm. *Drawida assamensis* Gates was the dominant earthworm species in the pineapple plantation soils (Tiwari *et al* 1989, 1990). Twenty numbers of intermediate size worms (Martin 1986) were thoroughly cleaned with sterilised water and each worm was cut into 3 parts; anterior (up to 4 cm) middle (from 4–8 cm) and posterior (from 8·0–12·5 cm) using sterilised scissors (Dash *et al* 1979).

The gut content of the different regions of the worms were collected in sterilised petridishes containing about 2 ml of earthworm ringer solution. Freshly produced worm casts were also collected aseptically.

Microfungal populations in earthworm gut contents and casts were estimated by the soil plate method (Warcup 1950) using rose bengal agar medium (Martin 1950). Approximately 0·015 g gut contents and casts were inoculated separately in sterilised petridishes, using a sterilised nichrome spatula. A few drops of sterilised distilled water were poured at the bottom of the petridishes to disperse the inoculum uniformly. Then approximately 15 ml molten and cooled (below 45°C) rose bengal agar, supplemented with streptomycin sulphate, was poured into the petridishes. The dishes (5 replicates) were gently rotated and incubated at a temperature of $25 \pm 1°C$ for 5 days and the fungi were observed under a binocular microscope for identification. The monographs of Raper and Thom (1949), Gilman (1957), Subramanian (1971), Barnett and Hunter (1972), Domsch *et al* (1980) and Ellis and Ellis (1985) were consulted for the identification of fungi.

3. Results and discussion

Nine genera of microfungi comprising 17 species were isolated from the gut contents and casts of the earthworm. The isolates were: *Alternaria alternata, Aspergillus nidulans, A. niger, Curvularia maculans, Fusarium moniliforme, F. solani, Mortierella ramanniana, Paecilomyces liliacinus, Penicillium chrysogenum, P. claviforme, P. fellutanum, P. funiculosum, P. javanicum, P. vermiculatum, Trichoderma koningii, T. viride* and *Torula herbarum* (table 1). Seventeen species were isolated from the gut (anterior 16; middle 12; posterior 10). Ten species comprising 6 *Penicillia*, two *Aspergilli*, one *Curvularia* sp. and one *Paecilomyces* sp. were isolated

Table 1. Percentage frequency of microfungi isolated from the gut contents and casts of *D. assamensis.*

Microfungi	Gut contents			Freshly laid worm casts
	Anterior	Middle	Posterior	
Alternaria alternata (Fr.) Keissler	6	6	—	—
Aspergillus nidulans Van dentatus	14	11	8	15
Aspergillus niger Van. Tieghem	25	19	20	28
Curvularia maculans Boedijn	—	—	8	10
Fusarium moniliforme Sheld	94	87	—	—
Fusarium solani (Mart.) Sacc	51	65	—	—
Mortierella ramanniana (Moller) Linnem.	8	—	—	—
Paecilomyces liliacinus (Thom) Samson	27	29	26	31
Penicillium chrysogenum Thom	83	88	85	90
Penicillium claviforme (Bain)	23	11	13	29
Penicillium fellutanum Biourge	40	44	40	41
Penicillium funiculosum Thom	31	36	14	30
Penicillium javanicum Van Beyma	21	15	13	19
Penicillium vermiculatum Dangeard	54	39	50	52
Trichoderma koningii Oudem	15	—	—	—
Trichoderma viride Pers. ex. Gray	62	—	—	—
Torula herbarum Pers. ex. Gray	4	—	—	—

—, Not isolated.

from freshly laid worm casts. Microfungal species viz., *A. alternata*, *F. moniliforme*, *F. solani*, *M. ramanniana*, *T. koningii*, *T. viride* and *T. herbarum* were present in gut contents and were not recovered from the freshly laid worm casts. *C. maculans* was isolated from the posterior region and earthworm casts. The species of *M. ramanniana*, *T. viride*, *T. koningii* and *T. herbarum* were isolated only from the anterior region of the gut (table 1). This indicates that these microfungi are generally, digested in the middle region of the gut. *F. moniliforme*, *F. solani*, and *A. alternata* were isolated from the anterior and middle regions of the gut indicating that these fungi were digested in the posterior region of the gut of *D. assamensis*.

Present study demonstrated that maximum number of microfungi were digested in the anterior and middle region of the gut of earthworms. Similar fungal flora in posterior region of the gut and casts indicates that little digestion of fungal tissues occurs in the posterior region of the gut. This shows that a gradient exists with regards to the digestive capability of different regions of the gut of *D. assamensis* for utilization of microfungi; anterior > middle > posterior. *D. assamensis* grazed over microfungi and a reduced spectrum of fungal species was found in the casts. Dash *et al* (1979) in a similar study also recorded less number of microfungi in worm casts. In general, it may be concluded that earthworms are able to utilise a large number of soil fungi as food and it has a profound effect on the structure of microfungal communities of the soil.

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Morphological variation in *Gracilaria edulis* (Gmel.) Silva from the Mandapam region, India

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Abstract. *Gracilaria edulis* (Gmel.) Silva is represented by different morphological forms in the Mandapam region. The morphological variation of selected characters has been analyzed and quantified. Characters included basal branch constriction, branch endings, branch attenuation, stoutness of the thallus, angle of divergence of branches, branching index, sizes of medullary, subcortical and cortical cells, and gradation of cell size from cortex to medulla. All the characters showed continuous variation; however, branching index and branch attenuation showed significant negative correlation between them.

Keywords. *Gracilaria edulis*; natural population; morphological variation.

1. Introduction

Gracilaria edulis is the principal agarophyte resource of India and shows a good promise for its domestication. Hence, the phycocolloid content (Thomas and Krishnamurthy 1976; Mal and Subbaramaiah 1989) and ecology (Rama Rao and Thomas 1974; Shyam Sundar 1985; Mal and Subbaramaiah 1990a, b) have been studied.

G. edulis grows abundantly in and around the Mandapam region and has been harvested by commercial collectors over a period of two decades. Although the morphological variability in the Rhodophyta is widespread, systematic study of the phenomenon is limited. Morphological variation in *Pterocladia pyramidale* (Stewart 1968) and in *P. caerulescens* (Santelices 1978) has been studied. Chapman *et al.* (1977) described this phenomenon in a species of *Gracilaria* from the north Atlantic region, while Patwary and van der Meer (1982) extended the study to the wild type and mutants of *G. tikvahiae*.

The culture technology of *G. edulis* in the Mandapam region was developed by Raju and Thomas (1971). We are presently screening populations for a suitable strain of *G. edulis* capable of higher production under cultivation. To further this objective, morphological and anatomical variations of *G. edulis* occurring in four different localities around Mandapam were analyzed.

2. Materials and methods

Samples were collected in bulk during February-March, 1989, from four different places around Mandapam. They are: Rameswaram (lat. 9° 17′ N, long. 79° 19′ E), Pamban (lat. 9° 18′ N, long. 79° 13′ E), Thonithurai (lat. 9° 17′ N, long. 79° 11′ E) and Krusadai Island (lat. 9° 14′ N, long. 79° 13′ E). At Krusadai Island samples included one from the natural population in the lagoon water and the other from the

cultivated material grown in the lagoon. Mature plants were selected from the bulk sample after ascertaining their reproductive status under the microscope. In order to avoid the effect of seasonal variation on morphology and developmental sequence, samples were collected at the same time of the year.

Study of the phenotypic characters was based primarily on the methods outlined by Chapman *et al* (1977), Yamamoto (1978) and Patwary and van der Meer (1982)

2.1 *Basal branch constriction*

G. edulis shows a constriction at the point of origin of lateral branches. The degree of the constriction was found by measuring the diameter of (a) the constriction itself and (b) the branch before the next bifurcation, with a vernier caliper. The ratio of a/b provides an estimate of the degree of constriction. Measurement was taken for the 5 lowest branches of the plant.

2.2 *Branch endings and branch attenuation*

Four types of branch endings are commonly encountered: acute, acuminate, mucronate and obtuse (figure 1). The type of branch ending was recorded for each plant.

The degree of branch attenuation was measured by dividing the length by the breadth of the apices. Length was taken from the tip of the apices to the base before bifurcation and breadth was taken at the point of its greatest diameter.

2.3 *Stoutness of the thallus*

As the frond of *G. edulis* is circular in cross section, diameter of the main frond was measured at its thickest point.

2.4 *Angle of divergence of branches*

Five branches were cut from the lower region of the plant and the first angle from the basal part of each such branch was measured. In each branch, remaining
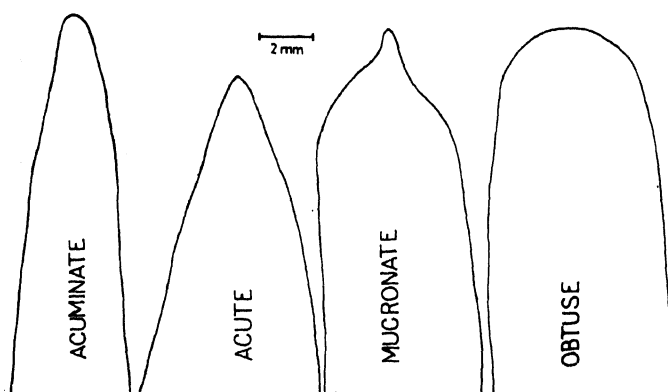
Figure 1. Types of branch apices.

branches above the angle which was to be measured, were excised to prevent the weight of the branches from distorting the angle. The angle between the branch stumps and the main axes was then measured by placing them on a herbarium paper previously marked with angles.

2.5 *Branching index*

Apical tips on thalli per gram of fresh weight were counted and expressed as the number per gram of material.

2.6 *Cell sizes*

Cell diameters were measured using a compound microscope, with calibrated ocular micrometer for cortical, subcortical and medullary cells viewed in transverse sections, made through the widest portion of each thallus. Except for isodiametric cells, the longest and shortest axes of cells were measured, summed and then averaged to yield a single value for the cell diameter. Fifteen measurements were taken for each of the cell types and a mean and standard deviation were calculated. In *G. edulis*, cell size gradually increase from cortex to medulla. Cortical and subcortical cells are arranged in rows, and variation in cell size within a row is low. Medullary cells occupy the major area of a section and there is enormous variation in size among the medullary cells. Hence, for medullary cells, only the largest cell was measured in each of 15 sections. Where the central portion of the medulla was occupied by small cells, an average was taken for 5 cells in each section. In contrast for cortical and subcortical cells, 15 measurements were taken using 3 sections. In each section, the diameter of 5 cells, arranged radially, was taken. In this way, both the variations within and among the sections were taken into account. Variation in the medullary region in sections of the fronds was drawn with the help of a camera lucida.

2.7 *Gradation of cell size from cortex to medulla*

The diameter of each cell, beginning from the cortex up to the centre of the medulla, arranged along an imaginary bisecting line, was measured. Increase in size of other cells over the cortical cell was calculated.

Besides the above characters, fresh weight and volume of the plants (determined by displacement) were also measured.

3. Results

The species *G. edulis* from the Mandapam region is represented in various morphological forms (figure 2). The value for the various characters ranged from 0·52–0·77 for basal branch constriction, 1·88–9·66 for branch attenuation, 1·5–3·0 mm for stoutness of the thallus, 49–96° for angle of divergence of branches, 100–402 for branching index, diameter 192·35–486·59 μm for medullary cells, 10·54–34·08 μm for subcortical cells and 4·77–7·66 μm for cortical cells. Measurements of the various phenotypic characters are presented in figure 3 (showing means and
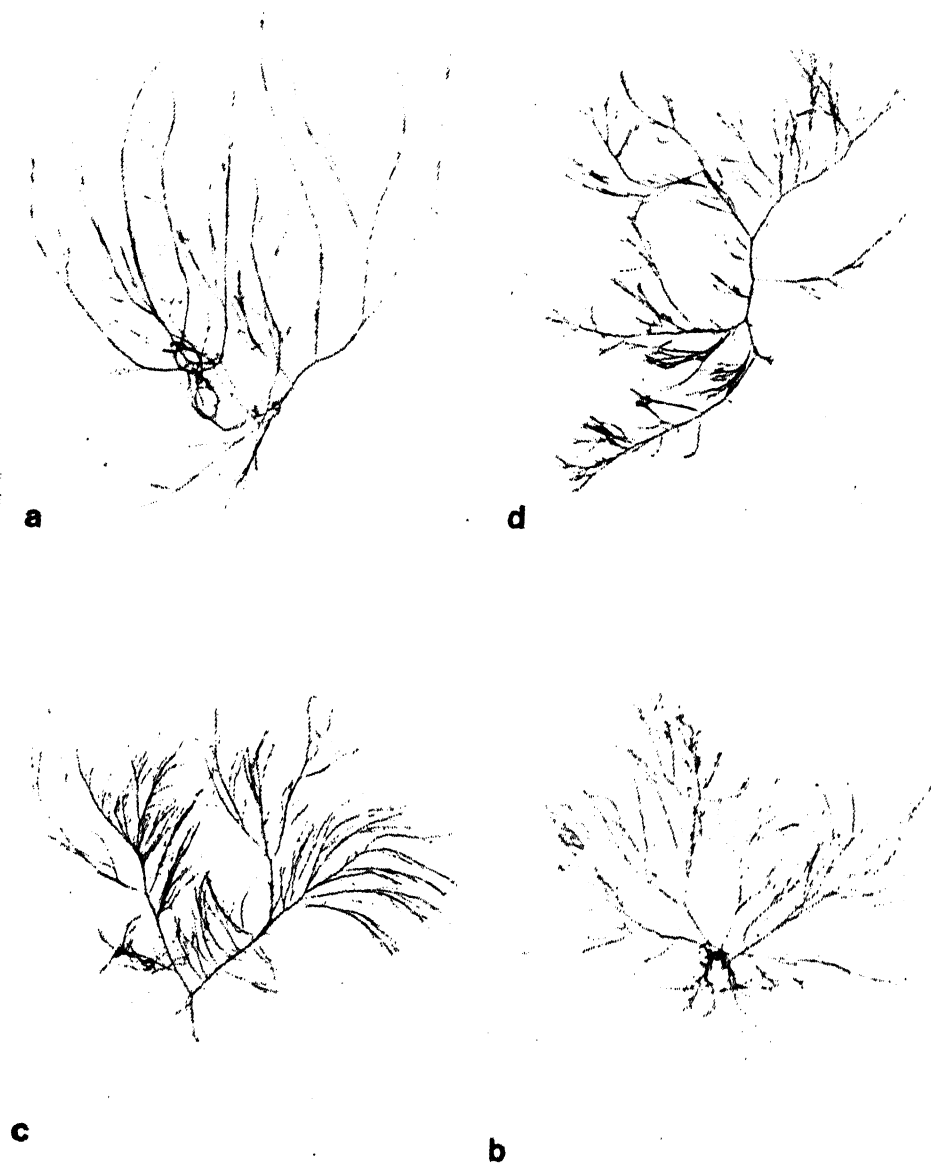
Figure 2. Representatives of *G. edulis* population with phenotypic variation. **a.** Plant with acuminate apices and low branching index. **b.** Plant with acute apices and high branching index. **c.** Plant with acuminate apices with high branching index. **d.** Plant with mucronate apices with low branching index (× 1/3).

standard deviations). Four distinct types of branch endings were noted: acuminate, acute, mucronate and obtuse of which acuminate and acute apices were most common. Sections through the thickest portion of the fronds also showed variation with gradual increase in cell size from cortex to medulla. In some cases, the
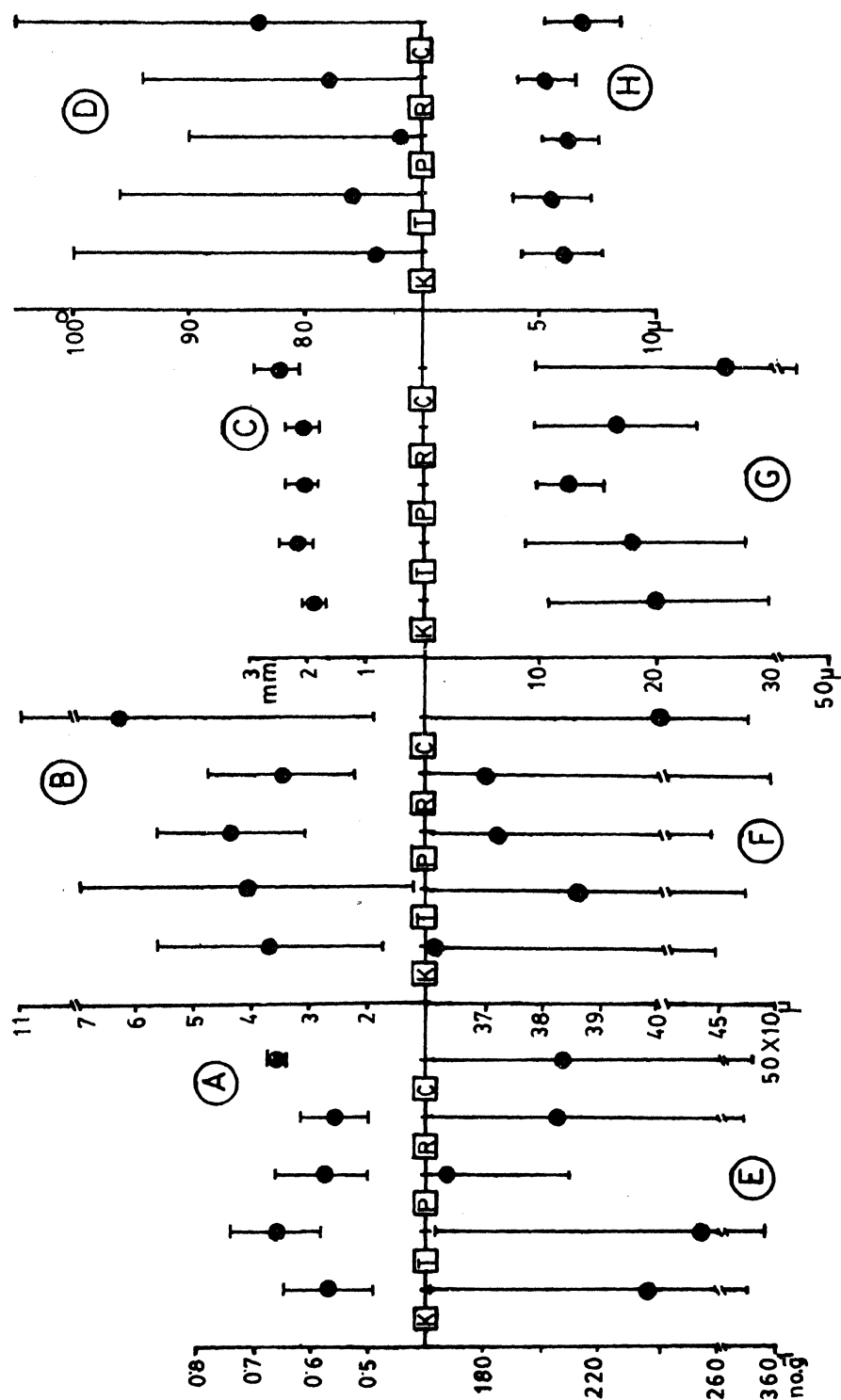
Figure 3. Graphical analysis of morphological and anatomical characters of *G. edulis* from different localities. **A.** Basal branch constriction. **B.** Branch attenuation. **C.** Stoutness of thallus. **D.** Angle of divergence of branches. **E.** Branching index. **F.** Diameter of medullary cells. **G.** Diameter of subcortical cells. **H.** Diameter of cortical cells. For each sample, mean ± standard deviation is shown. (K, Krusadai Island; T, Thonithurai; P, Pamban; R, Rameswaram; C, cultivated material at Krusadai Island).

innermost part of the medulla contained larger cells, with smaller cells in successively outer layers. In some plants the medullary region contained interspersed smaller cells. In others, the central portion of the medulla showed distinctly smaller cells than those in the outer medulla (figure 4a, b). To trace the nature of gradation in cell sizes from cortex to medulla, the increase in cell size in successive layers over that of the cortical cell is plotted in figure 5. The number of concentric cell layers generally varied from 5–8. However, it occasionally reached 12, especially where the central medulla was occupied by smaller cells.

In *G. edulis* all the characters exhibited overlapping values; but a significant negative relationship was found between branching index and branch attenuation (figure 6). The fitted regression equation in the scatter diagram is $Y = 6.889 - 0.012x$, $F = 7.0057$, $P < 0.05$ and $r^2 = 0.233$.

In the majority of cases the relative density was found to be unity except in a few cases in which it deviated slightly. This may be due to increase in their reserve food content.

4. Discussion

Although the species of *Gracilaria* exhibit morphological plasticity, variability in growth form has been studied in only a few species. Chapman *et al* (1977) characterised *Gracilaria* sp. from the lower Gulf of St. Lawrence and New England on the basis of basal branch constriction, branch attenuation, degree of thallus flattening, angle of divergence of branches and size of medullary, subcortical and cortical cells. To characterize *G. tikvahiae* mutants and wild plants, Patwary and van der Meer (1982) used the same characters in addition to stoutness of the thallus and branching index. To characterize *G. edulis* in the present study, the same characters were chosen, excepting the degree of thallus flattening, but incorporating
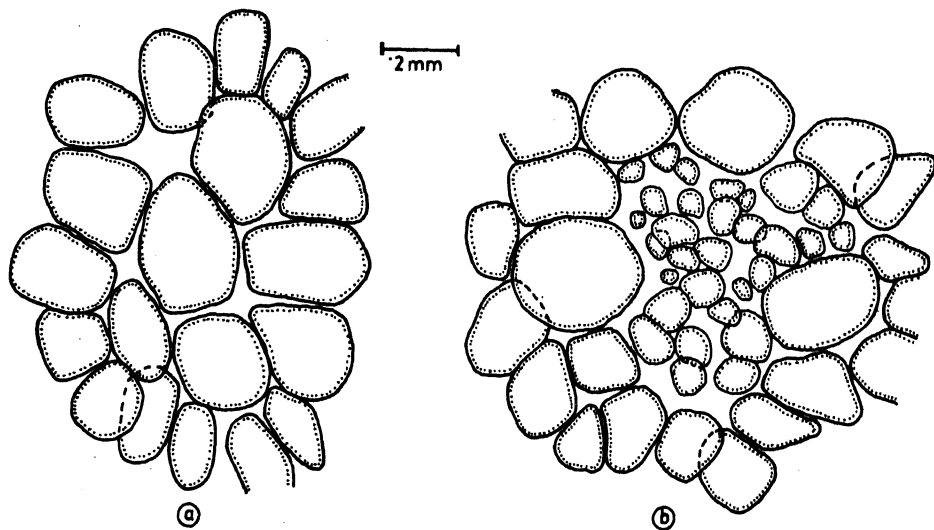
Figure 4. Sections through medullary region: Central medulla occupied by (a) larger cells and (b) smaller cells.
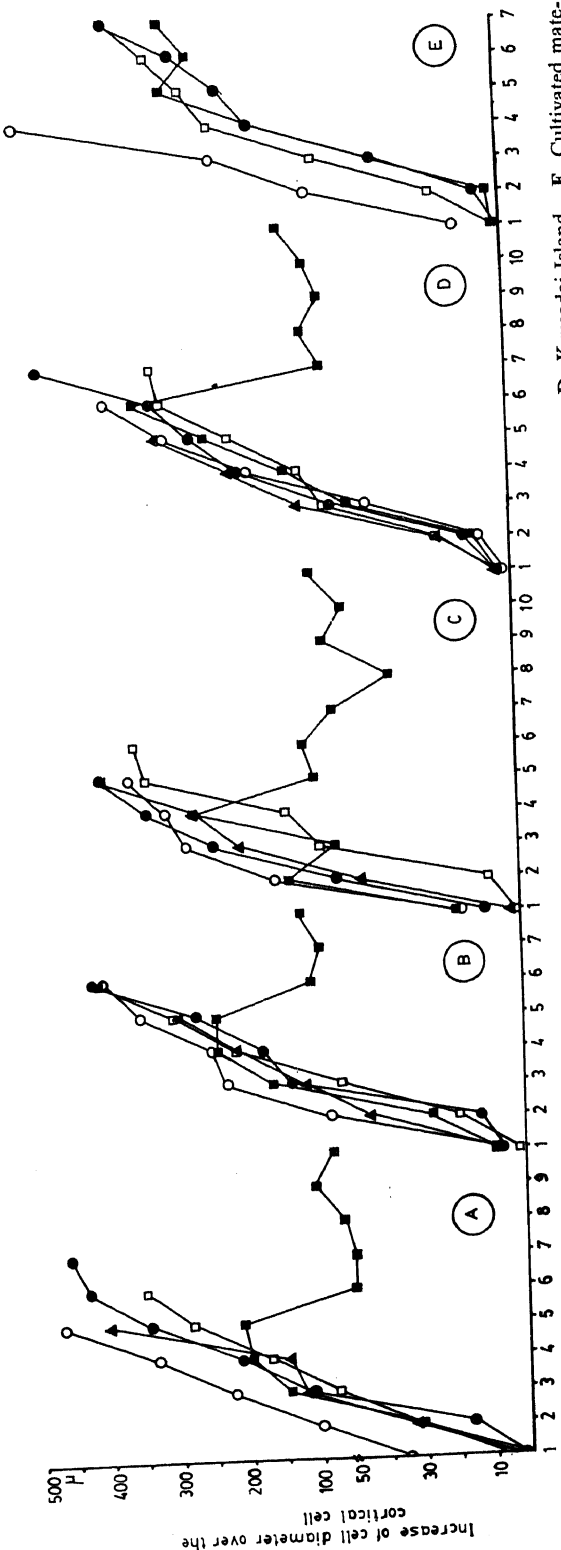
Figure 5. Gradation of cell size from cortex to medulla. **A.** Thonithurai. **B.** Pamban. **C.** Rameswaram. **D.** Krusadai Island. **E.** Cultivated material at Krusadai Island.
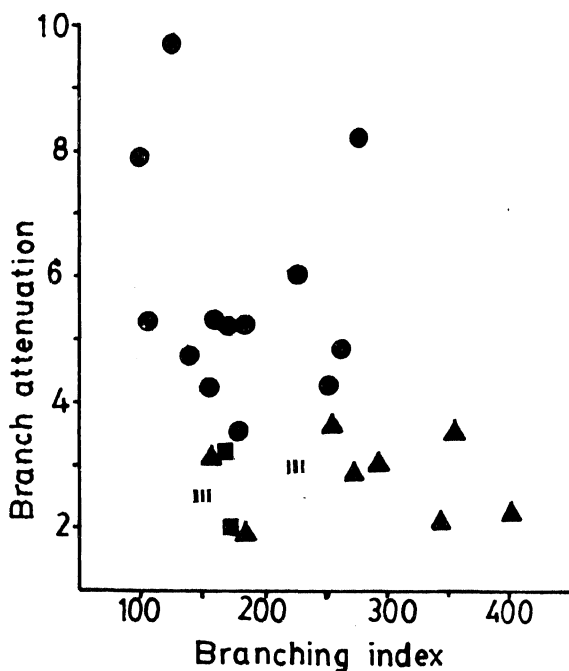
Figure 6. Relationship between branching index and branch attenuation of *G. edulis*. (●), Acuminate apices; (▲), acute apices; (■), obtuse apices; (Ⅲ), mucronate apices.

another character-gradation of cell size from cortex to medulla. Yamamoto (1978) used this character in his systematic and anatomical study of the genus *Gracilaria* from Japan. Measurement of branch attenuation was also made in a different way than that of Chapman *et al* (1977) where arbitrary code-numbers were given to designate the types of branch attenuation. Besides branch attenuation, types of branch endings were also recorded. Acuminate apices have a higher degree of branch attenuation in comparison to other type of branch endings. However, the values obtained from the present study for various characters are compared with those of the earlier work on other species of *Gracilaria* (table 1). There is little variation in basal branch constriction among *Gracilaria* sp., *G. tikvahiae* and *G. edulis*; but the angle of branching is rather higher in the latter two. *G. edulis* also has a much higher branching index than *G. tikvahiae*. The size of the medullary cell varies greatly and increases from *G. tikvahiae* to *Gracilaria* sp. to *G. edulis*; but the size of the subcortical cells is greatest in the study of *Gracilaria* sp. than in the other species. The smallest cortical cells were found in *G. edulis*.

It was observed that the size of the medullary, subcortical and cortical cells varied according to the stoutness of the thallus, while the number of cell layers remained nearly constant (figure 3). Higher angle of branching was also noticed with the stoutness of the thallus. The type of medulla containing smaller cells in fully grown and old fronds of *G. edulis* has been described earlier by Umamaheswara Rao (1972).

The variation of these morphological characters is difficult to describe in words (Patwary and van der Meer 1982) and is even more difficult to quantify, as values from each population overlap.

Table 1. Phenotypic plasticity in species of *Gracilaria*.

	Species		
	Gracilaria sp.	*G. tikvahiae*	*G. edulis*
Basal branch constriction	0·6–0·9	0·5–0·76	0·56–0·66
Angle of divergence of branching (degree)	40·00–64·00	51·81–87·39	72·20–84·00
Branching index (no. g^{-1} fresh wt.)	—	25·71–17·49	167–255
Cell size index (μm)			
Medullary	140–225	69·69–89·75	60·83–399·92
Subcortical	35–70	16·21–27·27	12·49–26·23
Cortical	9–14	9·65–12·63	5·26–6·79
Reference	Chapman *et al* (1977)	Patwary and van der Meer (1982)	Present study

The morphological variations that were encountered in this study were not found to be related to habitat, as the various phenotypes could be found in the same geographic locale. Similarly the same type of morphology was found in different localities. It is clear, therefore, that morphological variants cannot be described as ecotypes.

Patwary and van der Meer (1982) successfully employed branching index and frond stoutness as the principal characters to distinguish several discontinuous groups in *G. tikvahiae*. Chapman *et al* (1977), on the other hand, found all the characters to be overlapping in *Gracilaria* sp. The present study also shows the continuous variation of all the characters with a significant negative correlation between branching index and branch attenuation. However, as both branching index and branch attenuation also show overlapping values, it is difficult to arrange the populations into various discontinuous groups.

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Reproductive biology of *Plantago* L. III. Floral adaptation to wind pollination in *Plantago lagopus* L.

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Abstract. *Plantago lagopus* L., a weed belonging to family Plantaginaceae, has several features that allow its pollen to be easily drifted by wind. The syndrome of anemophily sensu Faegri and van der Pijl is represented in this species. In addition, some individuals in all populations are male sterile, and all plants contain dimorphic pollen within their pollen sacs. Pollen dimorphy ensures the dual requirement of dispersal over long distances and easy trapping by the plumose stigma.

Keywords. Anemophily; male sterile; *Plantago* L.

1. Introduction

Genus *Plantago* L., based on 282 species (Pilger 1937; Craven 1976; Briggs 1980; Sykes 1988) is the largest of the 3 genera included in the family Plantaginaceae. It is interesting on account of the variation exhibited by its species in their breeding system. The outbreeding rates vary between 0–100% among the different species of the genus (Wolff *et al* 1988).

The predominantly outbreeding species are characterised by a syndrome of characters involving different organs, many of these are represented in *P. lagopus* too.

2. Materials and methods

Plants of *P. lagopus* were raised in the University Botanic Garden during November 1988 and 1989. Different aspects of the floral biology were studied at regular intervals. Stigma receptivity was checked through the pollen germination test. For this purpose, pistils of different ages were fixed in acetic alcohol (1:3) and stained in a mixture of 2 ml 1% aq. acid fuchsin, 2 ml 1% aq. light green, 40 ml lactic acid and 46 ml distilled water. Stigmas having germinating pollen grains attached to their papillae were considered receptive.

The number of pollen grains per flower was estimated by counting the number per anther and multiplying this figure by the number of anthers present within the flower. Pollen count divided by the number of ovules per flower led to estimates of pollen-ovule ratio. For every attribute of morphology and floral biology about 10–30 values were recorded.

3. Results

Plants of *P. lagopus* have their lanceolate leaves adpressed to the soil surface, in the form of rosettes (figure 1) and they average 58 cm in height (table 1). The flowers are

Figure 1. A plant of *P. lagopus*.

borne in spikes which are carried on long scapes. The number of inflorescences is around 72 per plant. The length of spike/scape at the beginning and end of anthesis averages 1·85/35·11 and 5·49/48·6 cm, respectively (table 1).

Flowers are compactly aggregated in spikes ($\bar{x} = 149 \pm 21$ per inflorescence). They are small, odour-less and without any nectar. Each flower is tetramerous (figure 2). The petals are fused at the base forming a 3·6 mm long, tube like structure (table 1).

The flowers are protogynous; the stigma protrudes 4–5 days before the flower opens (figure 3). It turns receptive on the day of its emergence or a day later and remains in this phase for 4–5 days. Even before anthesis stigmas carry considerable pollen loads ($\bar{x} = 59$ per stigma), 80% of which is seen germinating.

The ovary is bilocular with one ovule per locule. The elongated ($\bar{x} = 5.76$ mm long) plumose stigma is clothed with prominent papillae (figure 4). Anthesis initiates with the expansion of petal lobes at the tip and emergence of stamens. The filaments measuring 5·8 mm on the average carry anthers well above the floral tube (table 1).

The anthers on an average are 2·6 mm long, versatile and filled with large quantities of pollen. The pollen-ovule ratio per flower averages 17,920. The pollen is dry with a smooth wall and dimorphic (figure 5). The pollen grains differentiating within the same anther lobe vary between 13 and 27 μm in diameter. The pollen is broadly classified into two size groups viz., big and small. The difference between the two pollen types is significant at 0·01 probability level. The frequency of the two types per anther is 70 and 30% respectively (table 1).

Male sterile individuals are quite common within the species. They have only

Table 1. Morphological characters of *P. lagopus*.

Parameter	Sample size	Value
Plant height (cm)	20	57·8 ± 2·7
		(45·1–70·6)
Height of spike/scape at (cm)		
Initiation of anthesis	22	1·85 ± 0·65/35·11 ± 1·95
		(1·2–1·7/18·0–41·5)
End of anthesis	19	5·49 ± 0·3/48·6 ± 1·59
		(4·2–7·5/41·3–57·3)
Height of spike and scape above the leaf whorl (cm)	12	39·77
		(32·2–48·4)
Length of floral tube (mm)	27	3·64 ± 0·07
		(3–4)
Length of stigma (mm)	31	5·76 ± 0·10
Length of filament (mm)	29	5·81 ± 0·11
Pollen count/anther	14	8960
		(7,058–10,612)
Pollen-ovule ratio/ flower	14	17,920
Pollen size (µm)		
Small	198	15·03
Big		23·38
Percentage frequency (%)		
Small	—	29·53
Big		70·47

shrivelled anthers which may or never come out of the flowers. The stigmas of male sterile plants are much longer than those of their fertile counterparts (figure 6). They keep on growing until pollinated with compatible pollen.

Estimates of pollen loads on the stigmas of male sterile individuals have revealed the dominance of pollen of smaller size. The frequency of such pollen grains is greater on stigmas of the male sterile plants located at a distance from the pollen source (table 2). Stigmas of fertile plants bear both kinds of pollen grains. In keeping with their higher percentage in anthers, the large sized pollen grains dominate in numbers even at the stigmatic surface.

Plants of this species are also visited by insects viz., *Apis dorsata*, *Apis florea* and some dipteran flies.

4. Discussion

P. lagopus has several features that favour pollination by wind. The syndrome of anemophily consist of long scapes, extruded and versatile anthers, long plumose stigmas, large quantities of dry and small pollen grains and above all protogyny. Abundance of pollen inside the anthers attracts *A. dorsata*, *A. florea* and some dipteran flies to the flowers. They come in search of pollen for consumption or as
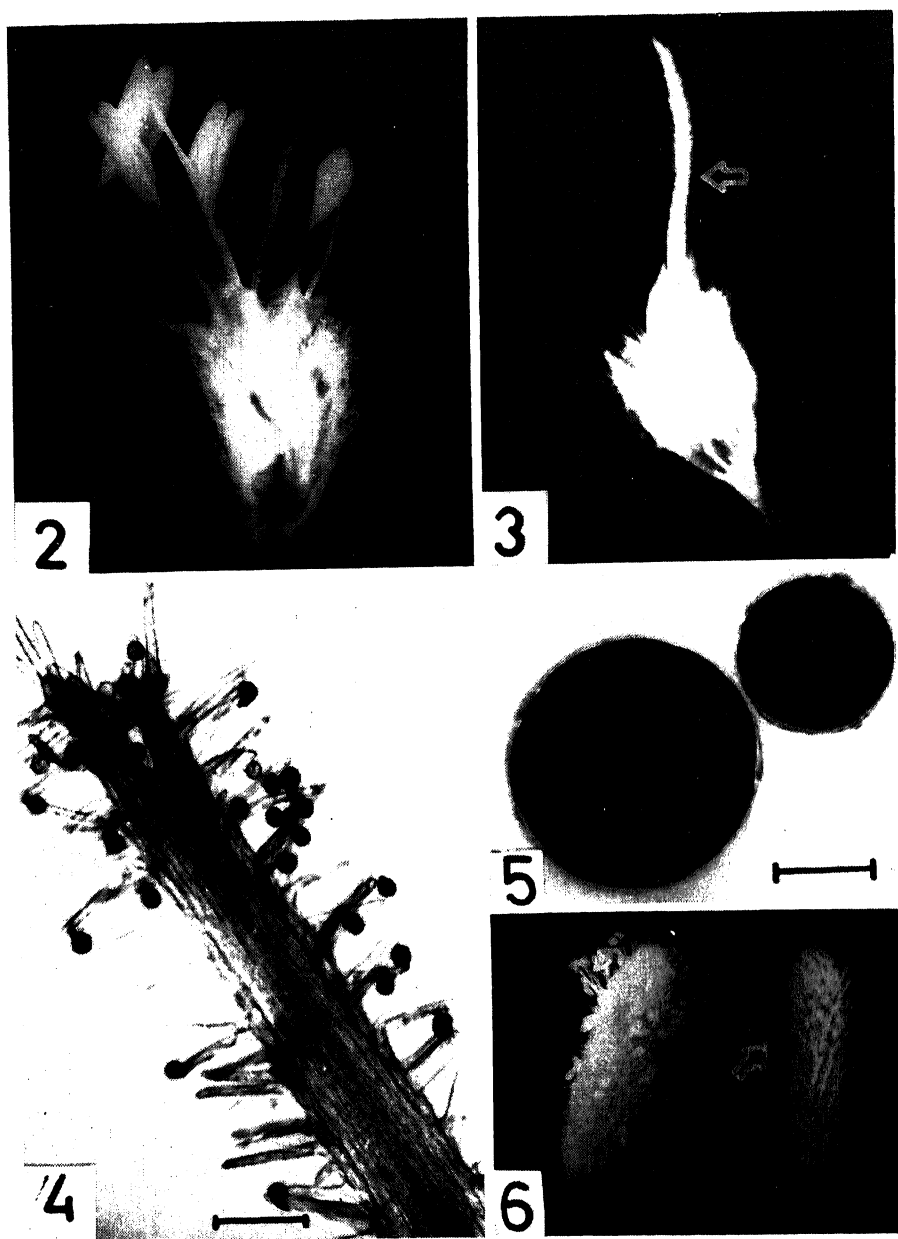
Figures 2–6. **2.** A mature flower of *P. lagopus* with extruded stamens and the stigma (×10). **3.** A flower one day prior to opening (×11). Note the elongated plumose stigma. **4.** A portion of plumose stigma of a male sterile individual bearing germinating pollen all over (Bar = 10 μm). **5.** Dimorphic pollen grains (Bar = 10 μm). **6.** Inflorescences of a male fertile and male sterile (see, arrow) individuals of the species. Note the highly elongated stigmas and absence of anthers in the latter.

food for their brood. The frequency of their visits and their behaviour on the spike indicate that they play little role in pollination. Insect visits to the inflorescence start only with the beginning of anther dehiscence. The visits are restricted to the

Table 2. Data on pollen load and percentage frequency of different pollen types on stigmas of male sterile plants.

Distance from pollen source	Number of stigmas studied	Pollen-load* on stigma	Break up of the two types of pollen grains	
			Big	Small
0·65 m	23	34·43	41·35%	58·65%
0·91 m	20	9·53	31·82%	68·18%
1·17 m	20	4·35	24·68%	75·32%

*Average number of pollen grains present on stigma.

regions of the inflorescence which bear dehiscing anthers. In flowers where anther dehiscence is in progress and in those located a few whorls immediately above these, the stigmas are already pollinated with pollen sufficiently ($\bar{x} = 59$ per stigma) to sire the two ovules present within the ovary.

Flowers of some other chasmogamous species of *Plantago* are also wind pollinated (Sagar and Harper 1964; Primack 1978; Bos *et al* 1985). Male sterility discovered in *P. lagopus*, also exists in *P. ovata*, *P. coronopus* and *P. lanceolata* (Atal 1958; Van Damme 1983, 1984; Van Damme and Van Delden 1982; Bos *et al* 1985; Wolff *et al* 1988).

Production of small, dry pollen grains of uniform size in large numbers is an important feature of anemophilous plants (Faegri and Van der Pijl 1979). Such pollen has advantage in dispersal as it can drift over long distances. The differentiation of differential sized pollen grains by a single plant, like *P. lagopus*, however, demands explanation. Referring to the relative importance of big and small sized pollen grains in wind pollination, Whitehead (1969) proposed that both these types satisfy diverse aerodynamic requirements. Small pollen grains disperse well but are not easily trapped by the stigma. On the contrary, the large pollen grains have high terminal velocity resulting in safe-settlement. Differentiation of diverse sized pollen grains in *P. lagopus* can thus be regarded as an additional adaptation for successful wind pollination, by fulfilling the dual needs of dispersal over long distances and easy trapping by stigmas.

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Vascular morphology of stipe and rachis in some western Himalayan species of *Pteris* Linn.

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Abstract. Vascular supply to leaf in 8 species of the fern genus *Pteris* is described. Except in *Pteris cretica* in which the stipe is supplied by a pair of ribbon like vascular bundles, stipe vasculature of the other 7 species studied is solitary and gutter-shaped; in transection the vascular bundle in *Pteris cretica*, *Pteris dactylina* and *Pteris stenophylla* is V-shaped, Ω-shaped in *Pteris wallichiana* and horse-shoe shaped in others. In *Pteris vittata* and *Pteris wallichiana* pinna trace is extra marginal in origin while in all others it is marginal. Based on number and structure of vascular strand and nature of origin of pinna traces it is concluded that *Pteris cretica* and *Pteris vittata* are relatively advanced over other species with simply pinnate fronds. *Pteris wallichiana* has been considered as highly evolved among the species investigated.

Keywords. Adaxial xylem plate; leaf vasculature; Ω-shaped vascular bundle; pinna trace; *Pteris*.

1. Introduction

Leaf vasculature in ferns is to a larger extent related with the morphology of the frond and it has also been considered as of significance in fern taxonomy (Ogura 1972; Lin and De Vol 1977). In addition to main vascular supply to frond, the primary pinna traces are also of taxonomic significance (Bower 1926). Apart from (being helpful in fern) taxonomy, the leaf vasculature can be useful in tracing phylogenetic relationships within various groups (Bower 1926). While describing the rhizome morphology of *Pteris indica* var. *integrifolia* Bedd. and *Pteris wallichiana* Ag. Tansley and Lulham (1904) and Mehra (1944) also made mention of the structure of leaf trace in these ferns respectively. Although some aspects of vascular structure of stipe of some Indian species of *Pteris* have been discussed by Chandra and Nayar (1970) and Khare and Shankar (1989), available literature on stipe anatomy of ferns (Tansley and Lulham 1904; Tansley 1907, 1908; Sinnot 1911; Davie 1918) reveal that there is no detail information on the leaf vasculature of the polymorphic genus *Pteris*. In view of this the leaf vasculature in 8 species of *Pteris* was studied.

2. Materials and methods

Fresh materials of all the 8 species (table 1) of *Pteris* collected from different localities of Pithoragarh district of Kumaon (western Himalaya) was fixed in FAA (1:1:3) for 48 h and then stored in 70% ethyl alcohol. Anatomy of stipe and rachis was studied from microtome and/or hand sections stained with safranin and fastgreen. Stelar reconstructions are based on serial sections cut at 8–10 μm. Special attention was paid to the general form and shape of the vasculature and

Table 1. Characteristics of leaf vasculature in 8 species of *Pteris*.

	P. cretica	P. dactylina	P. stenophylla	P. vittata	P. excelsa	P. quadriaurita	P. biaurita	P. wallichiana
No. of xylem strand	2	1	1	1	1	1	1	1
Size of xyelm strand at stipe base (mm)*	1×0·75	0·60×0·70	1×0·80	3·8×2·8	3·5×3·5	2·2×2·0	2·5×2·3	6·5×6·0
Shape of leaf vascu-lature in TS	V	V	V	U	U	U	U	Ω
No. of protoxylem groups								
At stipe base	2 in each str-and	3	3	14–16	20–24	13–16	12–13	44–50
At stipe apex	4	3	3	4–6	6–8	10–12	7–8	39–40
Formation of adaxial xylem plate	No	No	No	No	No	No	No	Yes
Nature of pinna trace	Marginal	Marginal	Marginal	Extra-marginal	Marginal	Marginal	Marginal	Extra-marginal
Shape of pinna trace	Flat	Flat	Flat	U	U	U	U	Ω

*Averages of 6 samples.

nature of pinnae traces. Voucher specimens are deposited in the Pteridology laboratory, Department of Botany, Government PG College, Pithoragarh, India.

3. Results

The leaves arise in spiral succession on the creeping or erect rhizome. Except *P. cretica* Linn., only one vascular bundle is present in the species investigated (table 1). In *P. cretica* two ribbon-like vascular bundles are present at the base of the stipe and both the margins of the xylem strand of each are incurved (figures 2, 21). The abaxial margins of vascular bundles are nearer to each other than the adaxial margins. Protoxylem elements are confined to both margins of each bundle. A little higher up in the stipe a few metaxylem elements are added marginally in the abaxial protoxylem groups of both the bundles which come closer and fuse with each other. The single vascular bundle thus comprises two marginal protoxylem groups in addition to two more in the abaxial arc. The vascular bundle in cross section is V-shaped (figures 4, 22). In specimens with short stipe the two vascular

Figures 1–14. **1.** Reconstruction of leaf vasculature in *P. cretica*. **2–7.** Successive stages of xylem strand in *P. cretica* (cross sections). **8.** Reconstruction of leaf vasculature in *P. dactylina*. **9–12.** Successive stages of xylem strand in *P. dactylina*. **13.** Shape of xylem strand (in cross section) at the stipe base in *P. vittata*. **14.** Reconstruction of leaf vasculature in *P. vittata*. **15.** Reconstruction of leaf vasculature in *P. stenophylla*. **16.** Reconstruction of leaf vasculature in *P. biaurita*. (blp, Basal lateral pinna; lp, lateral pinna; s, stipe; tp, terminal pinna).
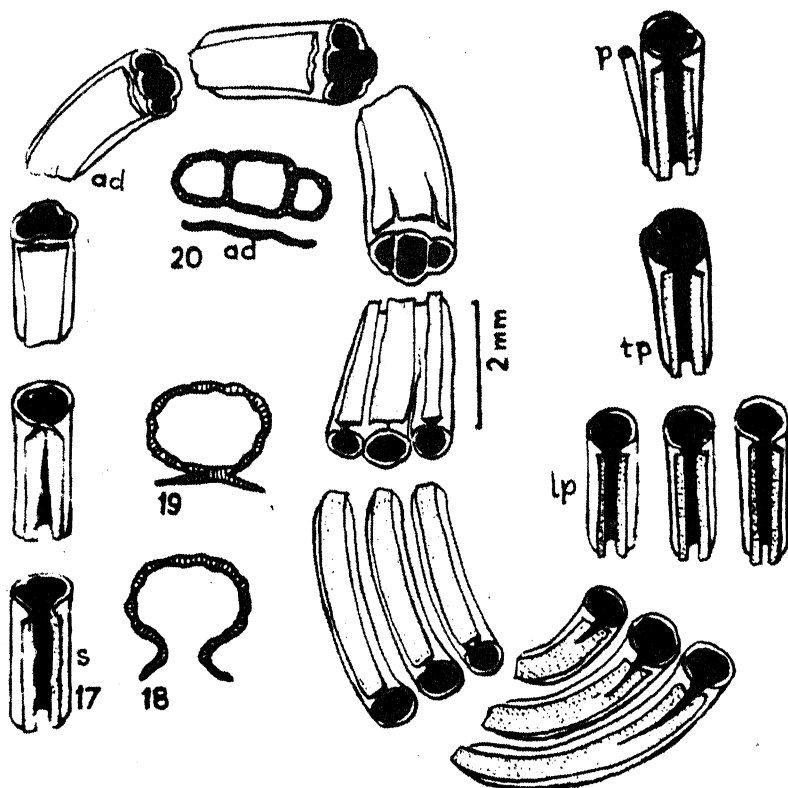
Figures 17–20. 17. Reconstruction of leaf vasculature in *P. wallichiana.* **18–20.** Successive stages of xylem strand in *P. wallichiana* (cross sections).

bundles unite at the base of the stipe (two distinct vascular bundles arise from the rhizome), in some others the union of bundles took place almost half way up in the stipe, in still others (especially in long stiped fronds) they unite farther up in the stipe but definitely before the departure of the pinna trace. Nearer to stipe apex the adaxial protoxylem elements of each arm increase in number which mark the formation of the pinna trace to basal pinnae. About 1–1·5 cm behind the basal pair of pinnae an abstriction appears next to the free adaxial margins of each arm of the protoxylem group which deepens gradually and from each arm is given off marginally one pinna trace (figures 1, 5) which supplies the basal pinnae on that side. In few specimens the united drain-like main vascular bundle passes upward in the rachis and splits into two strap shaped vascular bundles which, however, reunite before giving off pinnae traces to the second pair of the lateral pinnae (figures 1, 6, 7). In other specimens the main bundle does not split after supplying to basal pair of lateral pinnae and only one bundle is maintained throughout, from which the pinnae traces are given off successively and ultimately a shallow drain-like bundle enters the terminal pinna.

Only one vascular bundle supplies each leaf in *P. dactylina* Hook. and *P. stenophylla* Wall. Three protoxylem groups, one each at the margins of free arms and the third abaxially at the place of union of two arms of V (in cross section of
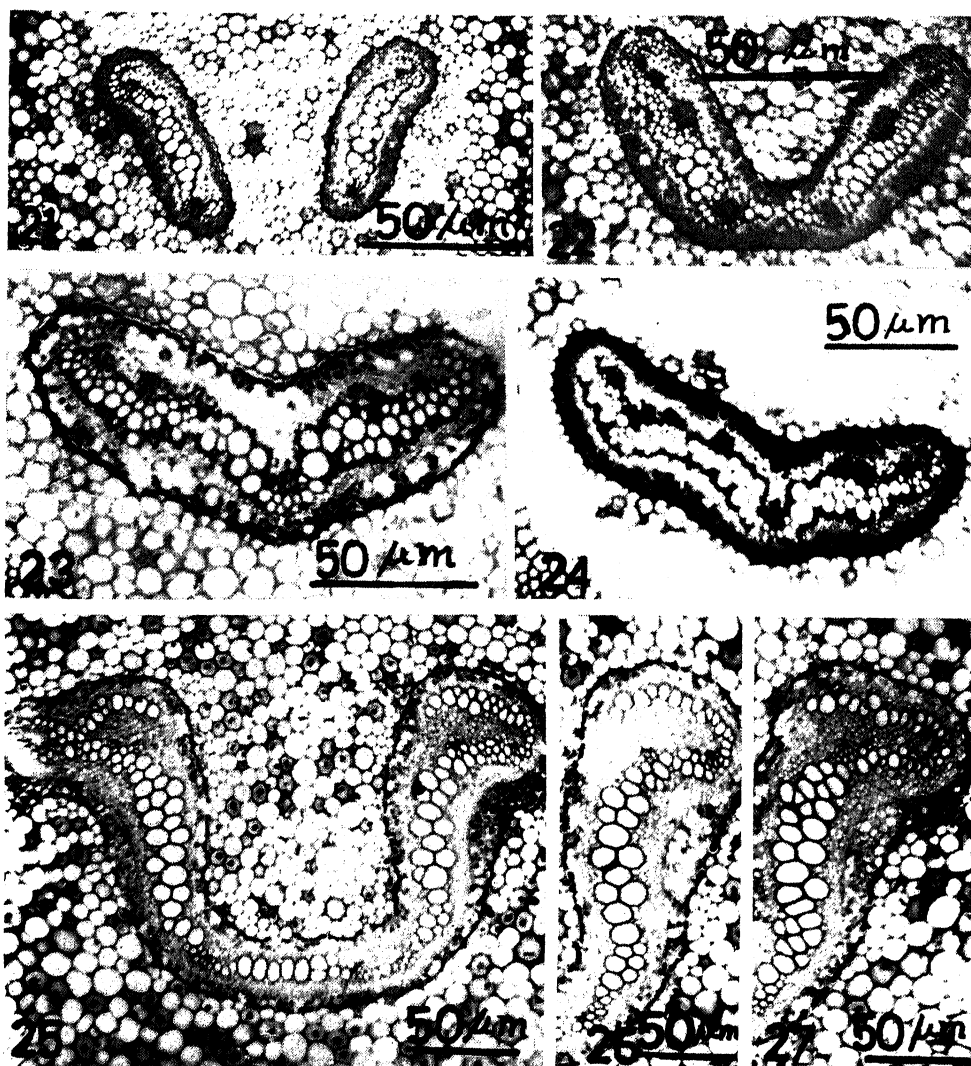
Figures 21–27. Vascular bundles in cross sections. **21.** *P. cretica*, showing binary leaf trace. **22.** *P. cretica*, fusion of two vascular bundles from their abaxial margins. **23.** *P. dactylina*, V-shaped vascular bundle prior to the departure of the pinna trace. **24.** *P. stenophylla*, V-shaped vascular bundle just before the separation of pinna trace (from the left arm). **25.** *P. vittata*, vascular bundle showing departing pinna trace extra-marginally (from the left arm). **26–27.** Successive stages of origin of extra-marginal pinna trace in *P. vittata*.

stipe the vascular bundle is V-shaped) remain unchanged throughout the length of the stipe. The marginal protoxylem groups are incurved and relatively more divergent in *P. dactylina* (figures 8, 9, 10, 23). Towards the stipe apex the marginal protoxylem elements increase in number and arranged in an oblique linear row to constitute the pinna trace. Thus, most of the marginal protoxylem elements are used up in building the pinna trace. The pinna trace which mainly comprises the

protoxylem elements is detached from the mother strand simply by an abstriction at the posterior part of the marginal protoxylem group (figures 11, 12, 15, 23, 24).

Relatively large gutter-shaped vascular bundle is found in *P. vittata* Linn. (figures 13, 25), *P. excelsa* Gaud., *P. quadriaurita* Retz. (figure 28) and *P. biaurita* Linn. (figure 16). Fairly large number of protoxylem groups alter with metaxylem groups in the xylem arc at the base of the stipe (table 1). This number is gradually reduced towards the stipe apex. The margins of the crescent at the base of stipe comprising metaxylem elements are incurved in *P. excelsa*, *P. quadriaurita* and *P. biaurita*. In *P. vittata* in addition to two protoxylem groups (separated by metaxylem), two more protoxylem groups are present in the hooked marginal part of the two arms (figure 25). Xylem elements at the extreme margins are larger than the normal protoxylem elements next to them. Up in the stipe, the sub-marginal protoxylem elements extend laterally, increase in number and are arranged in a circular ring (figures 25–27). This circular protoxylem group gradually separates from the mother strand and enters in the lateral pinna. In the meantime the marginal xylem elements rejoin the main strand. Thus, the pinna trace which mainly comprises the protoxylem elements originates extramarginally without the formation of any gap (figures 14, 27). New protoxylem elements develop behind the marginal metaxylem group which constitute the next pinna trace.

The large gutter-shaped leaf vasculature in *P. excelsa*, *P. quadriaurita* and *P. biaurita* generally remain unchanged except that it gradually narrows towards the stipe apex and the number of protoxylem groups are reduced in the process. The marginal protoxylem elements increase in number, extend adaxially somewhat obliquely in a linear row and separate from the mother strand by an abstriction to form the pinna trace. Pinna trace is gutter-shaped. For next pinna trace new protoxylem elements are added marginally.

Leaf vasculature in *P. wallichiana* is entirely different from other species examined in this study. Fairly large gutter-shaped (figure 17) vascular bundle arises from the
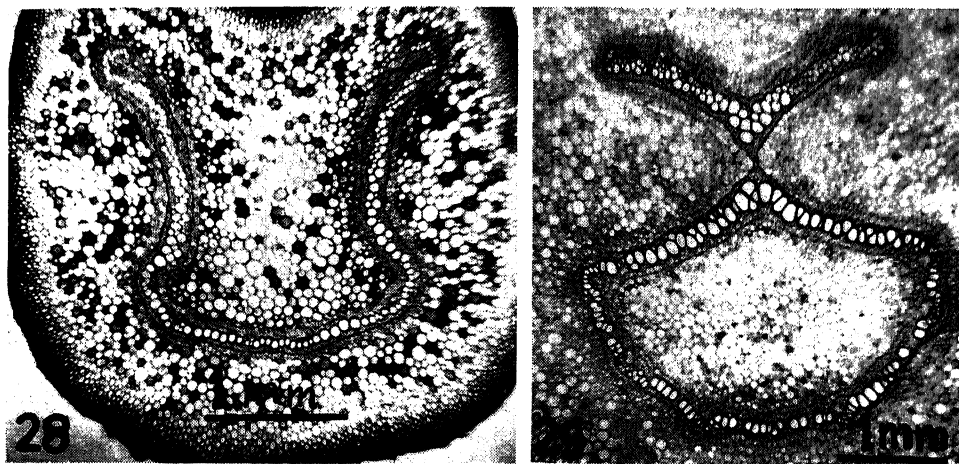
Figures 28–29. **28.** U-shaped xylem strand in *P. quadriaurita* (TS of stipe). **29.** TS of the base of terminal pinna of *P. wallichiana* showing re-union of adaxial xylem plate with corresponding circular main strand to supply the terminal pinna.

erect rhizome with the open end (in transection the vascular bundle is Ω-shaped) facing the adaxial side (figure 18). This configuration is maintained throughout the length of the stipe. At the stipe apex, however, both size and shape of the vascular bundle change remarkably which are noticeable about 2 cm behind the trifurcation (of the lamina). The vascular bundle becomes compressed along the invagination and the marginal flaps unite with each other, consequently an adaxial xylem plate is separated from the main bundle which now becomes circular (figure 19). From the circular bundle 3 daughter circular bundles are formed (figure 20). The adaxial xylem plate also splits into 3 almost equal parts by two abstrictions. One daughter adaxial xylem plate and a corresponding daughter circular bundle constitute the vascular bundle for one rachis branch (figures 17, 29). A median slit in the adaxial xylem plate make the vascular bundle Ω-shaped. Pinna trace originates partly from the bulged region of the vascular bundle and partly from the marginal flap of that side. The protoxylem group of the bulged region increase in number, extend laterally and make a circular ring, which soon separates from the mother bundle. At the same time the submarginal protoxylem elements of the corresponding marginal flap also increase in number. An abstriction in this region separates the marginal flap. The circular ring and the detached marginal flap together constitute a pinna trace. A median slit in the detached marginal flap make the pinna trace Ω-shaped.

4. Discussion

Fronds of *P. cretica*, *P. dactylina*, *P. stenophylla* and *P. vittata* are 1-pinnate; those of *P. excelsa* and *P. quadriaurita* are bipinnate and of *P. biaurita* is 1-pinnate and pinnatifid (commonly termed as bipinnatifid) whereas *P. wallichiana* is tripartite with each part pinnatifid. In *P. biaurita* a series of costal areoles and in *P. wallichiana*, a series of costular areoles in addition to the costal areoles is also present in each segment whereas the veins in rest of the species examined are forked. Anastomosing of veins and the presence of more than one leaf trace are believed to be advance characters over dichotomous veins and solitary leaf trace respectively. Within pinnate forms thus, it seems that *P. dactylina* and *P. stenophylla* are primitive and *P. cretica* and *P. vittata* are relatively advance. In *P. vittata* the pinnae traces are extramarginal, the character usually considered as advance (Bower 1923).

Vascular supply of the frond in *P. wallichianna* is interesting in that it is Ω-shaped and thus more advanced than in the other species studied. The size and shape of the leaf vasculature is associated with the general morphology of the frond. Among the species studied *P. wallichiana* bears the largest fronds. The nature of vascular supply to the lateral pinnae suggests that the basal lateral pair of pinnae are morphologically not similar to the lateral pair of pinnae of other species. Union of xylem strands at the invagination (Chandra and Nayar 1970) followed by separation of free arms of 'Ω' from the rest of the xylem to form an 'adaxial xylem plate' and formation of a circular xylem strand are unique features. Further, formation of 3 circular xylem strands from the main strand, breaking of the adaxial xylem plate into 3 parts and re-union of the adaxial plate (daughter) with its corresponding circular xylem strand leave no doubt that morphologically the lateral pinnae are identical to the terminal pinna. Almost identical vascular supply to fronds is known in gleichenioid ferns (Chrysler 1943, 1944; Punetha 1984).

Although no structure like adaxial xylem plate is formed in the leaf vasculature of gleichenioid ferns, formation of 3 circular vascular bundles at each fork is much alike. Of the 3 circular vascular bundles, the lateral two enter in the 'lateral rachis branches' whereas the middle one supplies the dormant apex.

Before defining precisely the branching of the leaf in gleichenioid ferns Holttum (1954) referred the laminar lateral pinnae as 'the lateral branches' and the terminal pinna as 'the middle branch' in *P. tripartita* Sw. (a species similar in branching of frond to *P. wallichiana*). Holttum (1957, 1959) coined the term 'rachis branches' to the lateral structures on the frond axes of gleichenioid ferns. The vascular supplies of the 'rachis branches' are similar to the vascular supply of 'main rachis' a situation very much similar to that of *P. wallichiana*. Further branching of terminal pinna and lateral pinnae is identical in *P. wallichiana* and *P. tripartita*. The lateral laminar structures in these species are morphologically and anatomoically identical to the terminal one. It is, therefore, not appropriate to use the term 'lateral pinnae' for these structures and should be termed as 'rachis branches' a term already in use.

It appears that the similarity in the leaf vasculature features is mainly on account of large leaves both in gleichenioid ferns (exceptions are the members of *Gleichenia* s.s.) and in *P. wallichiana*. Pinnae traces in the gleichenioid ferns are also extra-marginal and are given off from the side of the crescent. In *P. vittata* and *P. wallichiana* the pinnae traces are extra-marginal. In the former, however, the pinna trace arises submarginally whereas in *P. wallichiana* the pinna trace arises from the bulged region behind the invagination which is comparable with the pinna trace in *Lophosoria* (cf Bower 1923). Bower (1926) considered extra-marginal pinna trace as derived and characteristic of ferns with large leaves.

The role of marginal and submarginal protoxylem elements in constituting the pinna trace is evident. In species with marginal leaf traces only marginal protoxylem elements participate whereas in *P. vittata* most of the submarginal protoxylem elements build the pinna trace. Although pinna trace in *P. wallichiana* is partly derived from the bulged region of the vascular bundle and partly from the marginal flap of that side, only protoxylem elements of these parts are used in the formation of extra-marginal pinna trace, a situation comparable with some species of *Hypolepis* (unpublished data).

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Cyperaceae Indiae Australis Precursores: A novelty in *Cyperus* Linn. and its vegetative anatomy

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Abstract. One set of specimens collected from higher altitudes of south India showing affinities with *Cyperus kurzii* Clarke is described as new species together with its vegetative anatomy. The anatomical characters of this novelty differ in several details from those of species hitherto known anatomically.

Keywords. Novelty in *Cyperus*: vegetative anatomy.

2. **Cyperus palianparaiensis** Govind. **sp. nov.** — Sect. **Diffusi** Kunth (figure 1)

Affinis *Cypero kurzii* C B Clarke sed culmis valde 7 costatis cum foliis minoribus ad numerum per culmum, foliis angustioribus plerumque brevioribus multinervis, bracteis brevioribus involucralibus radiis ad numerum minoribus rigidis, inflorescentiis minus densis spiculis majoribus ad numerum brevioribus latioribus per radium cum minoribus ad numerum floribus, glumis laxe distichis (semidistichis) purpureo-fuscis vel atrocastaneis obscure mucronatis cum inciso apice et enervibus lateribus, carina 3 nervata ut videtur 1 nervata fasciata sigmoidea, staminibus 3, antheribus maturis oblongis luteis cum tridentata crista, nucibus suborbicularibus late ellipticis vel cuneatis stipitatis 1/3 longitudinem glumorum cum depresso vel rotundato apice et dense subtiliter granulata pagina notabilis.

Govindarajalu 059, IIIrd Plot, High Wavys Mts., Madurai Dt., Tamil Nadu (type: CAL); paratypes: Govindarajalu 0106 A-B, Vattaparai, High Wavys Mts., Madurai Dt., Tamil Nadu: 0106 A (DD); 0106 B (BSI); Govindarajalu 14286 A-B, Palianparai, High Wavys Mts., Madurai Dt., Tamil Nadu: 14286 A (MH); 14286 B (BLAT).

Perennial. Roots few, thick, black. Culms prominently 7 ribbed throughout, thickened and woody at base, rigid, erect or flexuous, smooth, 25–75 cm × 2–3 (−3·5) mm. Leaves 1–2(−3) per culm, well developed, prominently multicostate, carinate, gradually acuminate, pale green, smooth margined, flat or canaliculate (15−) 18–35 cm × 4–5 mm; sheaths 1–3 lowermost bladeless, purplish brown or red covering culm bases, often shredded into fibrous strands; nerves purplish red; uppermost with short ovate lanceolate flat green blade and white membranous margin, 5–7 (−8) cm × 7–8 mm. Inflorescence usually decompound (compound) consisting of 3–6(−8) spikelets per ray, 5–10 mm across. Primary rays 5–8, usually 5-ribbed, erect or somewhat flexuous, 4–12 cm long; secondary rays usually 5-ribbed, 1–4 cm long, usually curved towards apex. Involucral bracts 3–5, leaflike, erect, unequal, longer than inflorescence, the longest up to 15 cm long, 3–3·5(−4) mm broad. Spikelets linear oblong or oblong ovate, angular, compressed, (8−) 10–16 flowered, approximate, spreading at ends of secondary rays, (4−) 5–7 × 2–3 mm; rachilla flexuous, persistent, excavated, winged. Glumes trullate, purplish brown or atrocastaneous, notched at apex, more or less compact, distichous (semidistichous)

Figure 1. **a–i.** *Cyperus palianparaiensis* Govind. *sp. nov.* **a.** habit (× 0·5). **b and c.** Nuts (× 20). **d.** Spikelet (× 8). **e.** Glume, lateral view (× 25). **f.** Glume, spread out (× 21). **g.** Rachilla, diagrammatic. **h.** Anther (× 50). **i.** Style with stigma (× 50) (from Govindarajalu 059, holotype).

with hyaline margin and nerveless sulcate sides, strongly keeled, mucronulate (acute), tannin streaked in the sulcus (sometimes) in the keel, 2 mm (excl. mucro) long and broad; mucro *circa* (c.) 0·25 mm long, recurved; keel strong, green, 3 nerved (seemingly 1 nerved and banded), sigmoideus, smooth. Stamens 3, usually exserted with flat membranous 1 nerved broad persistent filament; mature anthers yellow, oblong with tridentate crest, spurred at base, 0·5–0·6 mm long. Style 0·5–0·6 mm long; stigma 3, nearly 1½ times longer than style, sparsely papillate towards apex, often exserted. Nuts variable in shape, suborbicular, broadly elliptic or cuneate with rounded or depressed apex, triquetrous with flat or somewhat depressed sides, minutely apiculate, densely finely granulate, stipitate, dirty brown, 0·9–1 × 0·8–0·9 mm

Notes

(i) Readily recognizable features of this novelty in its habitats and herbarium are strongly 7 ribbed culms, presence of purplish brown or red broad bladeless ovate lanceolate lowermost sheaths with many red nerves, linear oblong or oblong ovate radiating spikelets at the ends of secondary rays and purplish brown or atrocastaneous glumes with nerveless sulcate lateral sides.

(ii) Instead of describing different type of ovate glumes under a general term 'ovate' in a traditional and uncritical way, the term 'trullate' is introduced here as the appropriate one to describe the particular ovate shape of the glume observed in this species (Stearn 1966; figure 1 f).

(iii) This novelty is less common and occurs in open places near the margin of rivulets and marshy places at c. 1,500 m.

Vegetative anatomy

Materials and methods: Bits of different organs were selected from the paratypes. The same methods and the descriptive terms followed in earlier works are adopted here (Govindarajalu 1966, 1968a,b, 1975; Metcalfe and Gregory 1964; Cheadle and Uhl 1948a,b).

Leaf—Abaxial surface: Intercostal cells axially elongated; cell walls thin, sinuous. Stomata (length 66·7–69·6 μm; width 34·8–37·7 μm), narrowly elliptic oblong; subsidary cells parallel-sided (figure 2d); interstomatal cells long with concave ends. Silica cells moderately long, broad occurring in 2–3 continuous rows each cell containing 3–4 cone-shaped silica-bodies surrounded by satellites.

Adaxial surface: Cells shortly hexagonal; cell walls thin, smooth. Stomata and silica cells, see abaxial surface.

T S Lamina (figure 2c). Width of specimen examined 4·2 mm. Outline 'T' shaped. Cuticle very thin. Keel elongated (length c. 1 mm long), distally bilobed. Margin abruptly acute. Hypodermis 1–2(–3) layers of polygonal translucent cells present both abaxially and adaxially. Sclerenchyma strands (length 34·8–46·4 μm; width 34·8–58·0 μm) pulviniform or rounded throughout. Air-cavities well developed, transversely elongated, large, regularly alternating with vascular bundles (vb's) each containing stellate parenchyma cells; keel with 3 vertically elongated air-cavities. Bulliform cells of single layer of 6–7 undifferentiated cells. Chlorenchyma and bundle sheaths as in group B of this genus (Metcalfe 1971). Vascular bundles 14; laminal vb's 11 and 3 in the keel; median vb inversely oriented; large keel vb (type III A); the remainder (type I); large keel vb containing protoxylem lacuna;
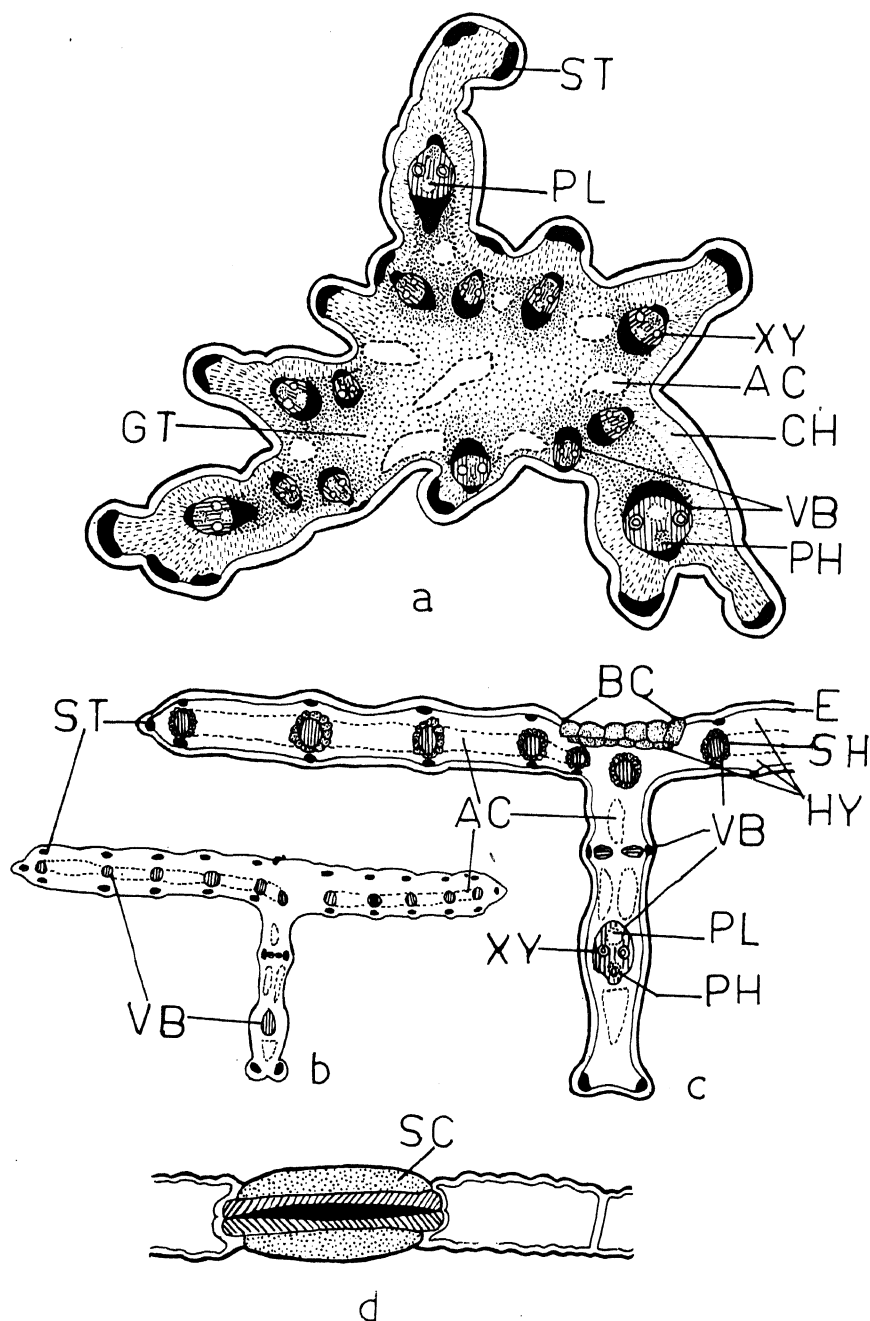
Figure 2. a–d. *Cyperus palianparaiensis* Govind. **a.** Transection of culm (× 55). **b.** Transectional outline of entire lamina, diagrammatic. **c.** A part of transection of lamina (× 44). **d.** Surface view of stoma (× 226) (all based on paratypes).
(AC, Air-cavity; BC, bulliform cells; CH, chlorenchyma; E, epidermis; GT, ground tissue; HY, hypodermis; PH, metaphloem; PL, protoxylem lacuna; SC, subsidiary cell; SH, bundle sheath; ST, sclerenchyma strand; VB, vascular bundle; XY, metaxylem).

metaxylem vessel elements (34·8 μm in diameter). Metaphloem of 'intermediate type. Secretory cells not common.'

Culm — Epidermis, surface view: Cells short, variable in size, hexagonal; cell walls thin, smooth. Stomata (length 58·0–65·7 μm; width 40·6–46·4 μm), elliptic oblong; subsidiary cells low dome-shaped; interstomatal cells short with concave ends. Silica cells not obvious.

T. S. Culm: (figure 2a): Width of the specimen along the longest axis *c.* 1 mm. Outline very irregular with 7–9 prominent ribs. Cuticle moderately thick. Epidermal cells large, isodiametric except over the ribs. Assimilatory tissue consisting of 2–3 layers of palisade chlorenchyma. Air-cavities *c.* 8 containing stellate prenchyma cells. Vascular bundles *c.* 14–15 forming a peripheral ring, those opposite to large ribs larger (type III A) than the remainder (type I); all vb's containing protoxylem lacunae. Metaxylem vessel elements (34·8 μm in diameter). Metaphloem of 'regular type'. Bundle sheaths and crescentiform sclerenchyma of vb's, see Metcalfe (1971). Sclerenchyma strands opposite to ribs crescentiform (pulviniform) (height 58·0 μm; width (92·8–) 116–174 μm). Central ground tissue consisting of large thin-walled cells tending to form central cavity. Secretory cells common in chlorenchyma.

Root. Transverse section: Diameter of root examined 0·9 mm. Exodermal cells single layered, variable in size; cell walls moderately thick. Cortex recognizable into 2 zones, outer broad, lacunose becoming net-like with several air-cavities, inner narrow containing 3–4 layers of compactly arranged cells. Endodermis: cells isodiametric, moderately thick-walled with broad lumen. Metaxylem central, circular; protoxylem units and metaxylem groups not distinct; metaxylem vessel elements (46·4 μm in diameter). Ground tissue parenchymatous.

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Cyperaceae Indiae Australis Precursores: New species and scanning electron microscopic observations in *Pycreus* Sect. *Muricati*

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Abstract. Three new species belonging to *Pycreus* Sect. *Muricati* are described and illustrated. The scanning electron microscopic studies of epicarpic surface markings reveal interesting details, real nature of the markings and differences among them. They are correlated with observations under binocular dissection microscope. It is now understood that transverse rugae and rugosely tuberculate condition of the epicarpic surface used as sectional characters seem to be nothing but the result of deposition of epicuticular wax. Interesting type of epidermal surface ornamentation is also recorded.

Keywords. *Pycreus*; epicarpic surface markings; SEM studies.

12. Pycreus apiculatus Govind. sp. nov. (figure 1)

Affinis *Pycreo zonatissimo* Chermez. sed culmis brevioribus densis, foliis non setaceis, culmis longis quam vel longioribus, bracteis plerumque 2 (−3) divaricatis reflexis, inflorescentia cum spiculis multioribus, spiculis acutis latioribus cum floribus multioribus, staminibus duo, nucibus orbicularibus transverse rugosis distinctis apiculatis 1/2 longis atque glumis differt.

Govindarajalu 15130, Devikulam, Lockart gap, Munnar, Kerala state, very common on roadside (*typus*: CAL); *paratypi*: *Govindarajalu 12107 A, B* Kulikad, High Wavys, Madurai Dt., Tamil Nadu: *12107 A* (BSI); *12107 B* (BLAT); *Govindarajalu 12086*, Kulikad, High Wavys, Madurai Dt., Tamil Nadu (MH); *Govindarajalu 12244 A, ibid.* (DD).

Annuals. Roots many, slender, yellow or brown. Culms few-many, caespitose or 1–3 subsolitary, thick (non filiform), erect, trigonous or subterete, leafy and woody at base, 5–10 (−12) cm tall. Leaves few per culm, smooth or distantly scabrid margined, non setaceous, as long as or longer than culms, flat, 1–1·5 mm broad; sheaths purplish red, many nerved, closed, transversely truncate. Involucral bracts 2–3, usually divaricately reflexed, sometimes distantly scabrid towards apex and obliquely erect or curved, the longest up to 7 cm long. Inflorescence simple, subspicate appearing capitate with (2 −) 5–8 spikelets. Spikelets ovate lanceolate or elliptic ovate, acute, remote, divergent, compressed or tumid, 15–18 flowered, sessile or shortly rayed, 6–12 × 3–3·5 mm. Rachilla erect, wingless, excavated, scaly, tannin dotted. Glumes broadly or suborbicular ovate, tightly distichous and overlapping, obtuse or subobtuse, purplish red, usually with narrow hyaline margin, 2–2·2 × 2 mm; keel green, 3-nerved. Stamens 2; filaments broad, usually persistent; anther yellow, oblong, minutely apiculate, 0·6–0·8 mm long; apiculus red. Style slender, 1–1·1 mm long; stigma usually included, *c.* 1 mm long. Nuts orbicular, distinctly apiculate, stipitate or sessile, 1/2 length of glumes, turgid, biconvex, black, transversely rugose, 1 × 0·8–1 mm.
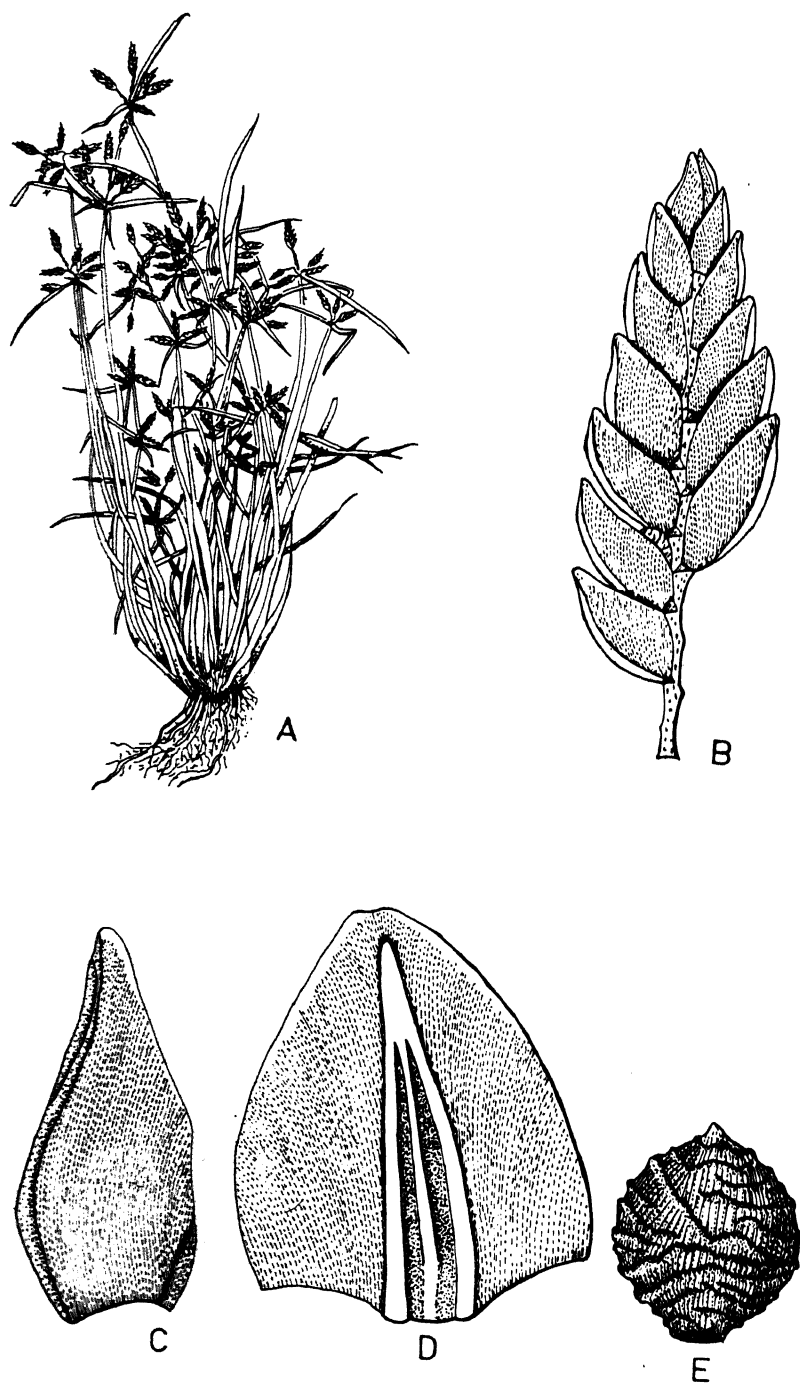
Figure 1. *Pycreus apiculatus* Govind. *sp. nov.* **A.** Habit (×1/2). **B.** Spikelet (×7·5); **C.** Glume, lateral view (×25). **D.** Glume, spread out (×21). **E.** Nut (×22·5) (from *Govindarajalu 15130, typus*).

13. Pycreus opulentus Govind. sp. nov. (figure 2)

Affinis *Pycreo paupero* (Hochst. ex A Rich.) C B Clarke sed culmis brevioribus rigidis foliis multis basalibus, foliis latioribus (non setiformibus) planis culmis longioribus, vaginis latis fuscis, inflorescentia capitata cum spiculis multioribus, bracteis brevioribus, spiculis ellipticis ovatis vel ovatis lanceolatis acutis cum floribus pluribus, glumis dense distichis uniforme atrocastaneis vel rubris, stigmate incluso, nucibus anguste suborbicularibus vel late ellipticis tuberculatis rugosis differt.

   Govindarajalu 11933, Thuvanam, High Wavys, Madurai Dt., Tamil Nadu (*typus*: CAL); *isotypi: 11933 A, B: 11933 A* (MH); *11933 B* (BSI).

Annuals. Roots many, slender, dark brown. Culms few-many, woody at base, caespitose, erect, rigid with many leaves at base, tricostate, 3–4 cm tall. Leaves many, acute, erect, flat, smooth margined, longer than culms, 2 mm broad (non setaceous); sheaths purplish red, open, many nerved, 3 mm broad. Inflorescence simple, capitate consisting of 4–5 spikelets. Involucral bracts (2 −) 3, leaflike, smooth or scabrid margined, short, up to 3·5 cm long. Rachilla flexuose, excavated, wingless, tannin punctate. Spikelets elliptic ovate or ovate lanceolate, acute-subacute, sessile, contiguous, compressed or subtumid, 8–12 flowered, 5–7 × 3–3·5 mm. Glumes broadly ovate, uniformly dark castaneous or red, compactly distichous, rounded or obtuse at apex with non hyaline margin, 2–2·2 × 1·8–2 mm; keel green, 3-nerved. Stamens 2; anther reddish yellow, linear, minutely apiculate, *c.* 0·75 mm long. Style long, slender, *c.* 0·6–0·7 mm long; stigma shorter than style, *c.* 0·25 mm long, included. Nuts narrowly or broadly elliptic or suborbicular, biconvex, turgid, black, apiculate, transversely rugose tuberculate, 1/2 length of glumes, almost sessile, 1 × 0·5–0·6 mm.

14. Pycreus plicatus Govind. sp. nov. (figure 3)

Affinis *Pycreo divulso* C B Clarke sed culmis plerumque solitariis rigidis erectis, foliis brevioribus culmis, bracteis plerumque 2 (− 3) divergentibus, inflorescentia capitata cum spiculis radiatis pluribus, spiculis contiguosis sessilibus ovatis lanceolatis acutis tumidis maioribus cum floribus pluribus, glumis late ovatis vel trullatis non mucronatis cum margine integro, rachilla cum margine sursus curvato vel plicato, nucibus obovatis vel suborbicularibus maioribus glumis parum minus longis atque differt.

   Govindarajalu 014125 A, Venniar to Varaiyattumottai, High Wavys, Madurai Dt., Tamil Nadu (*typus*: CAL); *isotypi: 014125 B–D: 01425 B* (MH); *014125 C* (BSI); *014125 D* (DD); *paratypi: Govindarajalu 11999 A–O*, Suruli R F, High Wavys, Madurai Dt., Tamil Nadu: *11999 A–E* (CAL); *11999 F, G* (BLAT); *11999 H, I* (BSI); *11999 J, K* (Assam); *11999 L, M* (DD); *11999 N, O* (MH).

Annuals. Roots few, slender, pale or yellowish brown. Culms usually solitary, sometimes 2–3, usually non caespitose or subsolitary, rigid, erect, thickened and leafy at base, trigonous, (4 −) 6–10 cm tall. Leaves usually shorter than culms, flat, setaceous, distantly scabrid margined towards apex, 1 mm broad; sheaths open, purplish red, many nerved; lowermost 1–2 sheaths bladeless. Involucral bracts 2 (− 3), usually divergent, as long as inflorescence, leaflike with scabrid upper half margin. Inflorescence simple, capitate consisting of 3–6 spikelets. Spikelets ovate
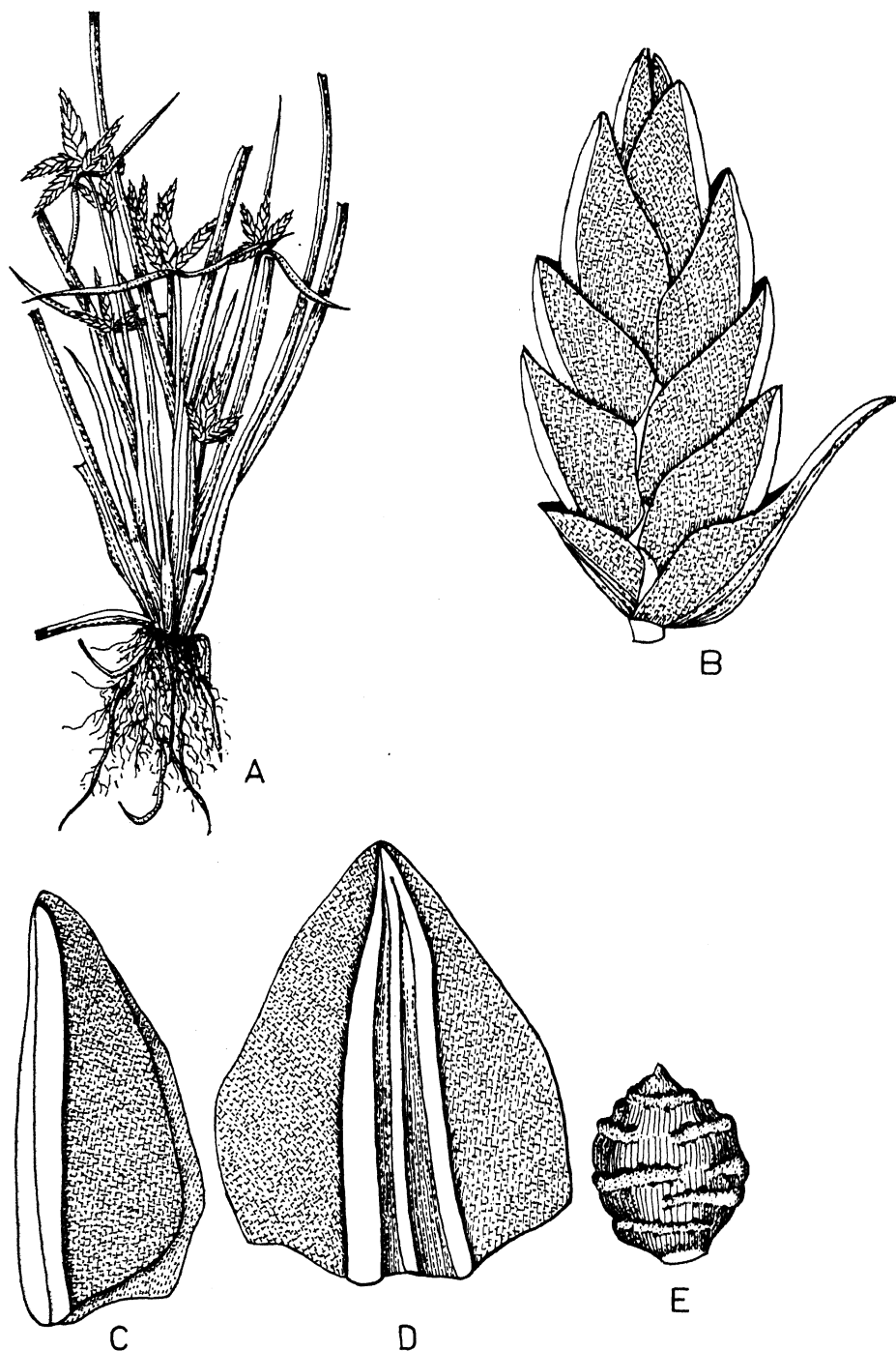
Figure 2. *Pycreus opulentus* Govind. *sp. nov.* **A.** Habit (× 2). **B.** Spikelet (× 10). **C.** Glume, lateral view (× 21). **D.** Glume, spread out (× 21). **E.** Nut (× 18) (from *Govindarajalu 11933, typus*).
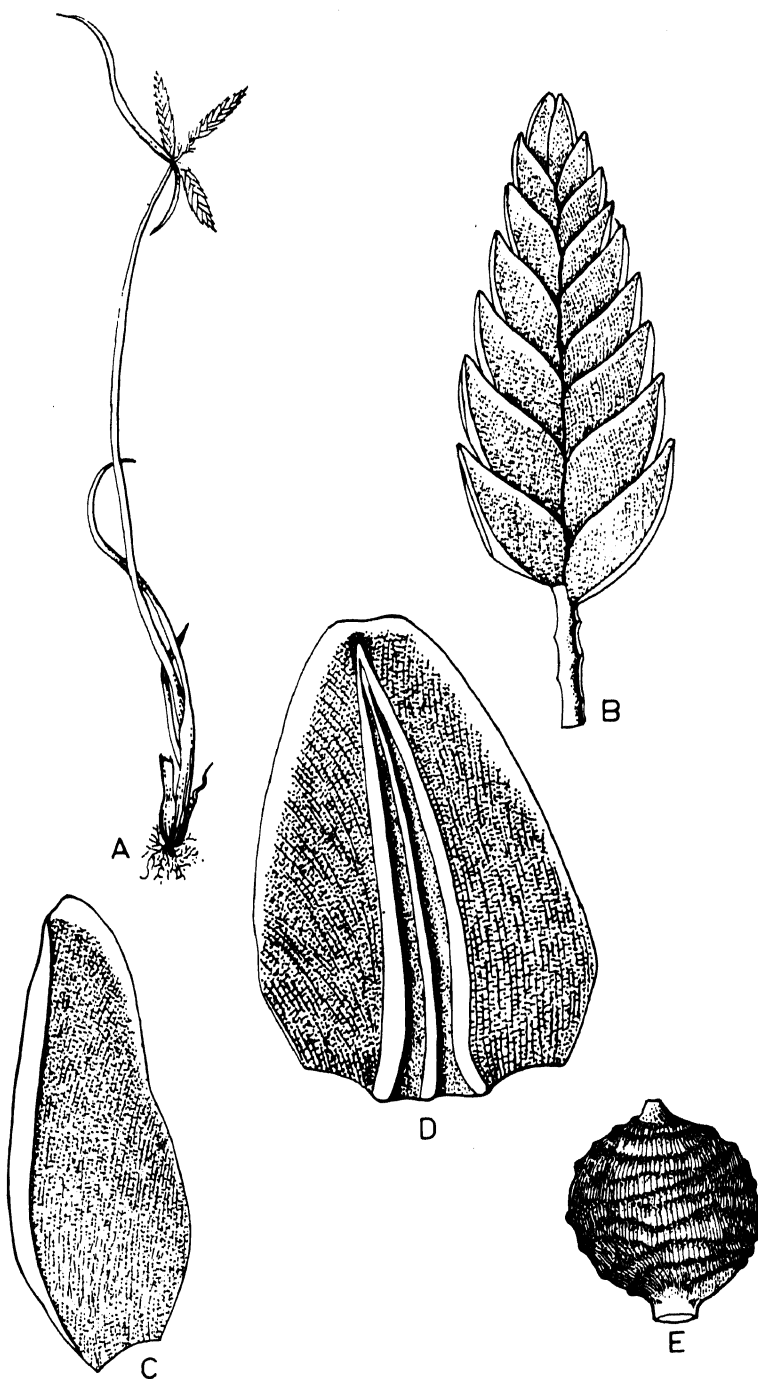
Figure 3. *Pycreus plicatus* Govind. *sp. nov.* **A.** Habit (×1). **B.** Spikelet (×7·5). **C.** Glume, lateral view (×27). **D.** Glume, spread out (×27). **E.** Nut (×21) (from *Govindarajalu 014125 A, typus*).

lanceolate, acute, 15–20 flowered, castaneous, tumid, 10–12 × 4 mm. Rachilla erect, excavated, wingless, tannin punctate with upcurved or folded margin. Glumes broadly ovate or trullate, compact, subacute or obtuse, dark castaneous brown, shining with narrow hyaline entire margin, muticous (non mucronate), 2·5 × 2 mm; keel green, 3 (– 5) nerved. Stamens 2 with persistent broad filaments; anther yellow, minutely apiculate, 0·9–1 mm long; apiculus red. Style short, slender, *c.* 0·25 mm long; stigma longer than style, usually exserted, *c.* 1 mm long. Nuts obovate or suborbicular, black, apiculate, stipitate, turgid, biconvex, transversely rugose tuberculate, little less than 1/2 length of glumes, shining, 1 × 0·9–1 mm.

SEM study

Materials and methods: From the mature nuts of the type specimens small fragments of the epicarp were removed from the mid portion by applying light pressure. They were mounted on metal stubs in dry condition using double sided tape and then coated with gold-palladium. With the help of JEOL T 330 A scanning electron microscope photographs of the selected areas of the specimens were taken.

Observations: In recent years SEM studies of utricles and/or nuts have been gaining impetus and importance in taxonomic works of Cyperaceae as repeatedly emphasized by several cyperologists (Schuyler 1971; Walter 1975; Toivonen and Timonen 1976; Bruederle and Fairbrothers 1983; Tallent and Weijek 1983; Standley 1985; Kukkonen and Toivonen 1988) though systematic investigations along these lines are very few. Recently Haines and Lye (1983) have given SEM pictures of entire nuts of east African rushes and sedges.

In different sections of *Pycreus* the outer surface of nuts is characterized by different patterns of surface markings specific to each one of them. These markings are either granulate ('punctate' *auct.*), quadrately or isodiametrically celled, longitudinally celled, transversely zonate or rugose and transversely rugose, tuberculate or muricate. Thus the epicarpic surface characters are undoubtedly considered to be useful as one of the sectional characters. Therefore SEM study is undertaken to find out the structural details and variability of the surface markings as revealed by SEM, to correlate the latter with what is observed under binocular dissection microscope and finally their taxonomic value. Likewise SEM study is proposed in future works on the remaining taxa.

Kükenthal (1936) has characterized the section *Muricati* by stating that the nuts are 'valde muriculato-rugosa' but the included species are said to be transversely 'tuberculato-rugosa' and 'muricato-zonato'. The taxa of this section described here have nuts with transversely rugose tuberculate markings.

The SEM characters common to the new taxa are (i) occurrence of epicuticular wax, (ii) dense aggregation and concomitant restriction of epicuticular wax over the . junction of epidermal cell rows which causes the transverse rugose condition and (iii) occurrence of erect minute spicular projections arranged in many more or less regular rows over the outer periclinal walls thus becoming part and parcel of the latter. Nevertheless the individual species differ from each other as follows: In *Pycreus apiculatus* the amount of epicuticular wax is relatively less and somewhat sparsely arranged plate-like or flaky masses. The anticlinal cell walls are tenuous
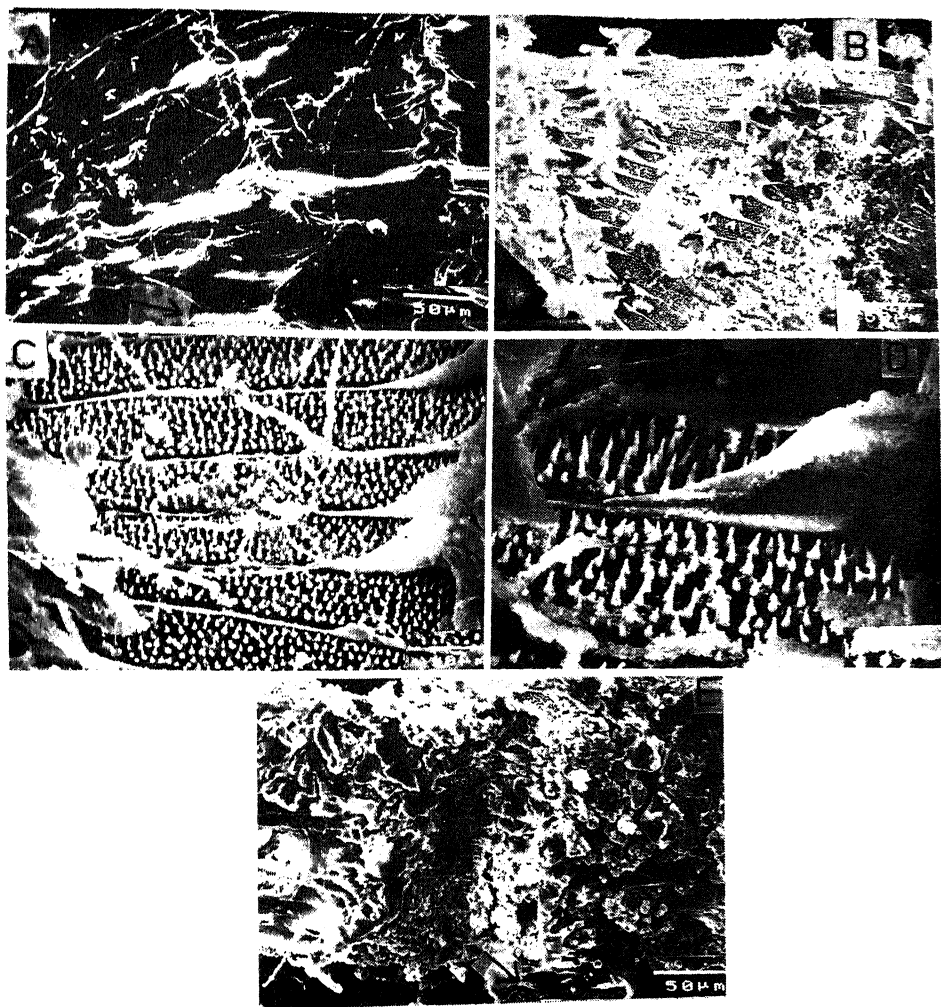
Figure 4. Epicarpic characters seen under SEM. **A.** *Pycreus apiculatus*, outer surface. **B–D.** *P. opulentus*, outer surface under different magnifications. **E.** *P. plicatus*, outer surface (all from type specimens).

and curved thereby rendering the entire epidermal surface undulate. The spicular projections of the cell walls could be observed only in a very few cells (figure 4A). In *Pycreus opulentus* the wax deposit is greater than in the former and also spreads both transversely and vertically coalescing with the adjacent ones to form a larger number of closely disposed flakes or platelets. The anticlinal cell walls are straight and conspicuous due to the extension of epicuticular wax. Unlike the situation in *P. apiculatus* and *P. plicatus* (see below) the spicular projections are conspicuously seen on the surface of every cell (figure 4B–D). In *P. plicatus* the epicuticular wax is so abundant that it camouflages the epidermal cells with the result the spicular projections could be observed only in a very few cells (figure 4E). Furthermore the epicuticular wax appears blob-like, convoluted and tubercle like (figure 4E).

In the light of SEM results it is plausible to conclude that the so-called 'rugose' condition seems to be the basic feature formed by the deposition of epicuticular wax at the junction of vertical rows of cells of these species but the tuberculate or muricate condition when present appears to be caused depending upon the varying pattern and quantum of wax deposition. The interesting spicular ornamentations also appear to be nothing but waxy excrescences which are more or less orderly aligned over the outer surface of periclinal walls. Furthermore the major common SEM characters mentioned above establish the homogeneity of the Sect. *Muricati* as well as the close relationships among the new taxa. These conclusions are also supported by the unpublished observations of other taxa belonging to the Sect. *Muricati.*

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Cyperaceae Indiae Australis Precursores: New species and combinations in *Pycreus* Beauv.

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Abstract. The status of *Pycreus* as an independent genus is confirmed. New combinations are proposed for two taxa originally considered as two distinct species of *Cyperus* and subsequently reduced to varietal status. They are described as two species of *Pycreus* based on new observations and illustrated. The species *Pycreus pumilus* (L.) Domin is considered *s. str.* and its revised synonyms and those of *Pycreus* are given. Two new species are reported in Sect. *Pumili* Kükenth. A key to the identification of the species is presented.

Keywords. New species: combinations: revised synonyms: key.

Pycreus

P. Beauv. Fl. Oware II: (1807), 48, t. 86, *pro gen.*; Nees in Linnaea IX: (1835) 483; C B Clarke in Hook.f. Fl. Br. Ind. VI (1893) 589 et in Kew Bull. add.ser. 8 (1908) 94 et Ill. Cyp. (1909) t. III and IV; C E C Fischer in Gamble, Fl. Madras 30: (1931) 1130; Hoper and Raynal in Kew Bull. 23 (1969) 513. -*Picreus* Juss. Dict. XL (1826) 194, *pro gen.* -*Cyperus subgen. Pycreus* (Beauv.) C B Clarke in Journ. Linn. Soc. XXI (1884) 33 and 35; Miq. Fl. Ind. Bat. III (1859) 254; Valck. Suring. Gesl. Cyperus Mal. Archip. (1898) 54; Kükenth. in Engl. Pflanzenr. Heft 101 (1936) 326; Blake in Journ. Arnold Arb. XXVII (1947) 220; Kern in Reinwardtia 3 (1954) 46 et seq. et in Fl. Males. 7(1974) 646; Koyama in Journ. Fac. Sci. Univ. Tokyo, sect. 3, Bot. 8 (1961) 37; Govindarajalu in Journ. Ind. Bot. Soc. 52 (1973b) 72 et in Proc. Indian Acad. Sci. 81 (1975a) 187; Haines and Lye in Sedges and rushes E. Afr. (1983) 268. -*Cyperus* sect. *Picreus* Griseb. Spicil. Fl. rumel II (1844) 419. -*Cyperus* sect. *Picreus* A. *Eucyperus* Boeck. in Linnaea XXXV (1868) 437; Benth. and Hook. Gen. Pl. 3 (1883) 1044. -subgen *Cyperus* L., Vahl, En. II (1806) 298; Nees in Wight Contr. (1834) 72; Kunth, En. II (1837) 3; Steud. Syn. Cyp. II (1855) 3.

It is evident from the above citation that *Pycreus* ever since its inception has either been treated as an independent genus or as a subgenus or section of *Cyperus s.l.* or indiscriminately intermixed with species of *Cyperus s. str.* without being ascribed any hierarchical segregation. Clarke (1884) has delimited *Pycreus* by "stylus bifidus, nucis compressae margo rachilla adjectus, nux fere asymmetrica." Furthermore he has stated on the basis of these clear cut characters that this is "the most natural and the most easily separable taxa" from all other subgenera included under *Cyperus*. Nevertheless he has somehow considered *Pycreus* only as a subgenus which procedure was followed by most of the cyperologists though some still considered it as a genus.

Van der Veken (1965) on the basis of embryographical evidences does not favour the splitting of *Cyperus s.l.* into a number of microgenera. Likewise from the anatomical information of the limited number of taxa hitherto studied it is not

possible at present to draw any definite conclusions about its correct taxonomic status (Metcalfe 1971; Govindarajalu 1978).

The above mentioned exomorphic characters namely the bifid style and the biconvex compressed (tumid) usually asymmetric nuts with characteristic surface markings (granulate or punctate, transversely zonate, rugose and/or muricate) are distinct and sufficient to consider *Pycreus* as an independent genus. In this respect it is but appropriate to consider this genus as 'morphogenus' just as morphologically differentiated species are called 'morphospecies' (Álvarez López (1957).

Section Pumili

Kükenth. in Engl. Pflanzenr. Heft 101 (1936) 375; Kern in Fl. Males. 7: (1974) 650. Type species: *Pycreus pumilus* (L.) Domin

Pycreus pumilus (L.) Domin in Bibl. Bot. Heft 85 (1915) 417; Turrill in Kew Bull. (1922) 124; C E C Fischer in Gamble, Fl. Madras 3 (1931) 1132; (errore 'pumilis'). -*Cyperus pumilus* L. Cent. Pl. II (1756) 6 et Amoen. acad. IV (1760) 302 et Sp. Pl. ed. 2 (1762) 69; Vahl, En. II (1806) 330; Kunth, En. II (1837) 4, *pro minore parte*; Miq. Fl. Ind. Bat. III (1856) 255; C B Clarke, Journ. Linn. Soc. XXI (1884) 43, non Rottb., nec Nees, excl. *f. membranacea*, var. *punctatus p.p. quoad pl. asiat.* et *Cyperus obstinatus*; C B Clarke, Fl. Br. Ind. VI (1893) 591; Valck. Suring. Gesl. Cyperus Mal. Arch. (1898) 55, t. II, fig. 10; Kükenth. Engl. Pflanzenr. Heft 101 (1936) 375, excl. var. *membranaceus, nervulosus*; Kern, Reinwardtia 3 (1954) 50 et Fl. Males. 7 (1974) 650. -*C. nitens* Retz. Obs. 6 (1789) 13; Vahl, En. II (1806) 331; Kunth, En. II (1837) 3; Nees in Hook. J. Bot. Kew Miscellany 6 (1854) 28; Steud. Syn. Pl. Cyp. II (1855) 3; Miq. Fl. Ind. Bat. III (1856) 255; Boeck. in Linnaea XXXV (1868) 483 incl. var. *capitatus*; C B Clarke in Hook. f. Fl.Br.Ind. VI (1893) 591 non Retz. -*C. pygmaeus* Retz. Obs. IV (1786) 9 (teste C E C Fischer in Gamble, Fl. Madras 3 (1931) 1132). -*C. pusillus* Vahl, En. II (1806) 303; C E C Fischer in Gamble, Fl. Madras 3 (1931) 1132. -*Pycreus nitens* (Vahl) Nees in Nova Acta Nat. Cur. XIX, suppl. I (1843) 53; C B Clarke in Hook. f. Fl. Br. Ind. VI (1893) 591; C E C Fischer in Gamble, Fl. Madras, 3 (1931) 1132. -*Cyperus pulvinatus* Nees et Meyen ex Nees in Wight Contr. (1834) 74; Thwaites, En. Pl. Zeyl. 2 (1856) 342. -*C. lemnoleptus* Steud. Syn. Pl. Cyp. II (1855) 3; Miq. Fl. Ind. Bat. III (1856) 255. -*Pycreus pulvinastus* Nees, Linnaea 9 (1835) 283; C B Clarke, Philip. J. Sci. Bot. 2 (1907) 79. -Figure 1a, b, i–1.

Pycreus membranaceus (Vahl) Govind. *comb. nov.* -*Cyperus membranaceus* Vahl, En. Pl. II (1806) 330; Kunth, En. II (1837) 3; Steud. Syn. Pl. Cyp. II (1855) 3; C B Clarke in Hook. f. Fl. Br. Ind. VI (1893) 591. -*C. pumilus* f. *membranaceus* (Vhal) C B Clarke, Journ. Linn. Soc. XXI (1884) 44. -*C. pumilus* var. *membranaceus* (Vahl) Kükenth., Engl. Pflanzenr. Heft 101 (1936) 376; Kern, Reinwardtia 3 (1954) 50. -*C. nitens* var. *membranaceus* (Vahl) Boeck., Linnaea 35 (1868) 484. -*C. obstinatus* Steud. Syn. Pl. Cyp. II (1855) 10. -Type Ind. Or., König (K). -Figure 1c–h.

Annual. Roots slender, many, dark dirty brown or black. Culms subsolitary or caespitose, gracile, strongly 3-ribbed (sometimes buried portions of culms rooting at nodes), (4 –) 10–20 cm × 0·5–0·6 mm. Leaves 1–2 (–3) per culm, gracile, flat, keeled, acuminate, scabrid towards apex, as long as or longer than or overtopping culms, 1–1·5 mm broad; sheaths stramineous or reddish brown. Inflorescence compound bearing up to 10–12 spikelets. Spikes ovoid or subsquarrose, 5–15 mm across.
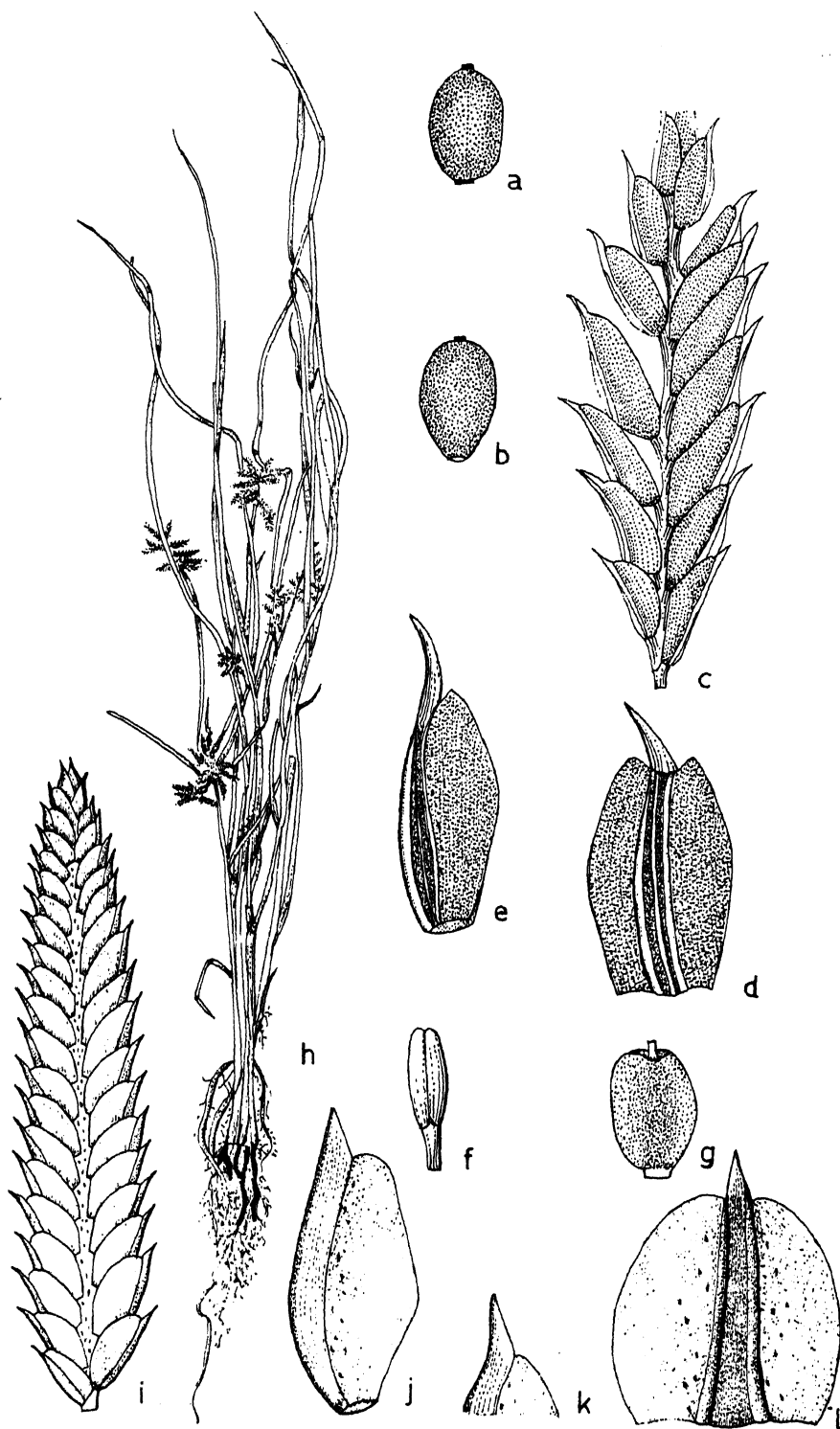
Figure 1. For Caption, see page no. 433.

Primary rays slender up to 4, 1·5–6 cm long. Involucral bracts usually 3 (− 5), leaf like, scabrid in upper margin, flexuous, the longest up to 18–20 cm long. Ochrea tubular, reddish brown, obliquely truncate, up to 5 mm long. Spikelets laxly spicate at ends of rays, oblong gradually widening towards apex, subacute, compressed, 6–18 flowered, 3·5–7 × 2·5 mm; rachilla flexuous, narrowly winged, excavated, persistent, visible, flat. Glumes oblong with more or less parallel nerveless sides, scarious sometimes tannin punctate, membranous, steeply oblique, laxly distichous, strongly keeled, mucronate, distinctly emarginate with 'V' shaped incision at apex, 1·5–1·6 (excl. mucro) × 1 mm; keel green, always 5-nerved; mucro recurved, 0·4 mm long. Stamens always 2 with short yellow anther with rounded apex, 0·1–0·2 mm long. Style slender; stigmas 2, as long as style. Nut oblong-elliptic, dark brown, apiculate, stipitate, minutely puncticulate, depressed or rounded at apex, sometimes asymmetric, 1/3 length of glumes, 0·7–0·8 × 0·4–0·5 mm; epicarpic cells minutely isodiametric appearing punctate.

Govindarajalu 6076, Sholayar, Kerala State (less common)—(CAL).

Notes

(i) According to Vahl (1806), Kunth (1837) and Steudel (1855) this taxon was considered as a distinct species characterized by gracile culms, leaves longer than culms, lax subsquarrulose spikes, linear smaller, 9--11 flowered spikelets, 5 nerved keel, single stamen (rarely 2 *sensu* Kunth, *l.c.*). Subsequently it was collected from Himalaya, Kumaon, Nepal, Bengal, Central India, Bombay, N. Kanara and Siam). Wall. Cat. n. 3312 B, C, G, H (microfiche !) agree well with the specimens studied here. Boeckeler, *l.c.* and Kükenthal, *l.c.* treated this as a variety the former under *Cyperus nitens* and the latter under *C. pumilus*. Clarke (1884) considered this as a form of *C. pumilus* stating as "Spicis magis laxis". Kern (1954) has pointed out that *C. pumilus* var. *membranaceus* (Vahl) Kükenth. does not agree with Kükenthal (1936) as the latter is supposed to have named rather slender specimens with longer many flowered spikelets only as a variety. Furthermore the type of *C. membranaceus* Vahl though seen by Kükenthal does not match with his description. On the other hand the characters as observed by Kern (1954) in the type also seem to agree with those of the author except in respect of longer culms (up to 20 cm instead of 8–9 cm) and longer rays (up to 6 cm instead of 1·5–2 cm). Nevertheless he concludes that they represent somewhat depauperated specimens of *C. pumilus* and that Vahlian concept of *C. membranaceus* has since changed considerably.

(ii) In spite of discrepant description and difference of opinion in the circumscription of this taxon the south Indian specimens not only agree with the characters given by Boeckeler (1868) and Kern (1954) which are based on Vahlian type but also resemble Wall. Cat. n. 3312 B, C, G, H (microfiche!) cited by these authors. Thus *C. membranaceus* differs from *C. pumilus* by taller gracile culms, narrower leaves as long as, longer than or overtopping culms, usually spicate inflorescence, longer rays, longer leaf-like bracts, elliptic ovate shorter divergent spikelets widening towards apex, lesser number of flowers (up to 20), oblong longer narrower glumes with more or less parallel sides and narrowly winged zig-zag visible rachilla, always 5-nerved keel, shorter curved mucro, always 2 stamens with shorter anthers and larger nuts. Because of the large number of distinct features which have nothing to do with the depauperated condition, *C. membranaceus* is considered as a distinct species.

Pycreus punctatus (Roxb.) Govind. *comb. nov.* -*Cyperus punctatus* Roxb. Fl. Ind.

1 (1820) 3; Kunth, En. 2 (1837) 3; Steud. Syn. Pl. Cyp. 2 (1855) 3; C B Clarke in Hook. f. Fl. Br. Ind. VI (1893) 591; Kükenth. in Engl. Pflanzenr. Heft 101 (1936) 375. -*C. pumilus* var. *punctatus* (Roxb.) Clarke in Journ. Linn. Soc. 21 (1884) 46.- Type (BM) (figure 2).

Annual. Roots many, fibrous, pale brown. Culms subtriquetrous, subcaespitose, stiff, filiform, 3–18 cm × 0·2–0·3 mm. Leaves flat or canaliculate, abruptly acuminate thus appearing filiform and coiled or flexuous (stiff and ensiform), scabrid towards apex, medianly keeled, as long as, little longer or shorter than culms, 0·2–1·25 mm; sheaths dirty brown. Inflorescence simple or compound usually with 1–2 rays added, spicate at end of rays or capitate when rayless. Bracts usually 3, leaf like, unequal, obliquely erect or flexuous (ensiform), scabrid towards apex, much longer than inflorescence, the longest up to 10 cm long. Ochrea obliquely truncate up to 1 cm long. Rays 1–2, obliquely erect, stiff, up to 4 (−5) cm long. Spikes ovoid or subglobose, dense with (5−) 15–20 spikelets, 1–2 cm across. Spikelets oblong ovate, spreading at right angles to rays or capitate and radiating, acute or subacute, subtumid, 15–30 flowered, 6–12 × 2·2–2·5 mm. Rachilla straight, wingless, tannin streaked. Glumes narrowly elliptic ovate or suborbicular obovate, membranous, lax oblique or almost erect (patent), strongly keeled with retuse or subacute apex and nerveless tannin streaked sides, stramineous white or rusty brown, 1–1·1 (−1·2) (excl. mucro) × 1 mm; mucro usually erect or incurved, 0·2–0·3 mm long; keel 3 nerved, green. Stamens 2 with persistent filaments; anther very small, yellow, oblong, 0·1–0·2 mm long. Style *c.* 0·5 mm long; stigma usually exserted. Nut elliptic, oblong ovate or obovate, usually rounded at apex, minutely apiculate, brownish black (black), densely granulate, shining, *c.* 1/2 length of glumes, 0·8–0·9 × 0·4–0·5 mm with granulate outer surface.

Venugopal 13933 A–B and 13934, Palani, Madurai Dt., (MH); Govindarajalu 6085, Sholayar, Kerala State (CAL); Waddod Khan 1380, Wakad (alt. 800′) and 1148 Sitakndi Bridge (alt. 1,800′) *sub* Fl. Bhokar and Hadgaon (BSI).

Notes

(i) *Cyperus punctatus* Roxb. was treated by Clarke (1884) as a variety of *C. pumilus* L. But it has several distinct characters namely subtriquetrous narrower culms, lesser number of rays, 3 bracts, sublax subtumid spikelets, narrowly elliptic ovate suberect very loose glumes, much shorter erect or incurved mucro, 2 stamens, larger nuts *c.* 1/2 length of glumes with rounded apex and granulate surface. Therefore *C. punctatus* is accepted here as a species and now placed under *Pycreus*.

(ii) Waddod Khan 1380 (*sub* Flora of Bhokar and Hadgaon; alt. 1,800′) is an interesting specimen because of its filiform culms, subcaespitose habit, pale white or stramineous appearance, much shorter leaves, lesser number of spikelets.

(iii) Wall. Cat. n. 3312 A, E (microfiche!) agree with the specimens cited above.

10. Pycreus palghattensis Govind. sp. nov. (figure 3)

Affinis *Pycreo squarrosulo* Chermez. sed ab eo differt culmis 3 costatis, inflorescentia perspicue spicata, bracteis 3, radiis brevioribus usque ad 4, spiculis pluribus ellipticis vel ellipticis ovatis remotis horizontaliter ad radios patentibus, spiculis parvioribus plus minusve tumidis laxe dispositis plerumque cum pluribus floribus,
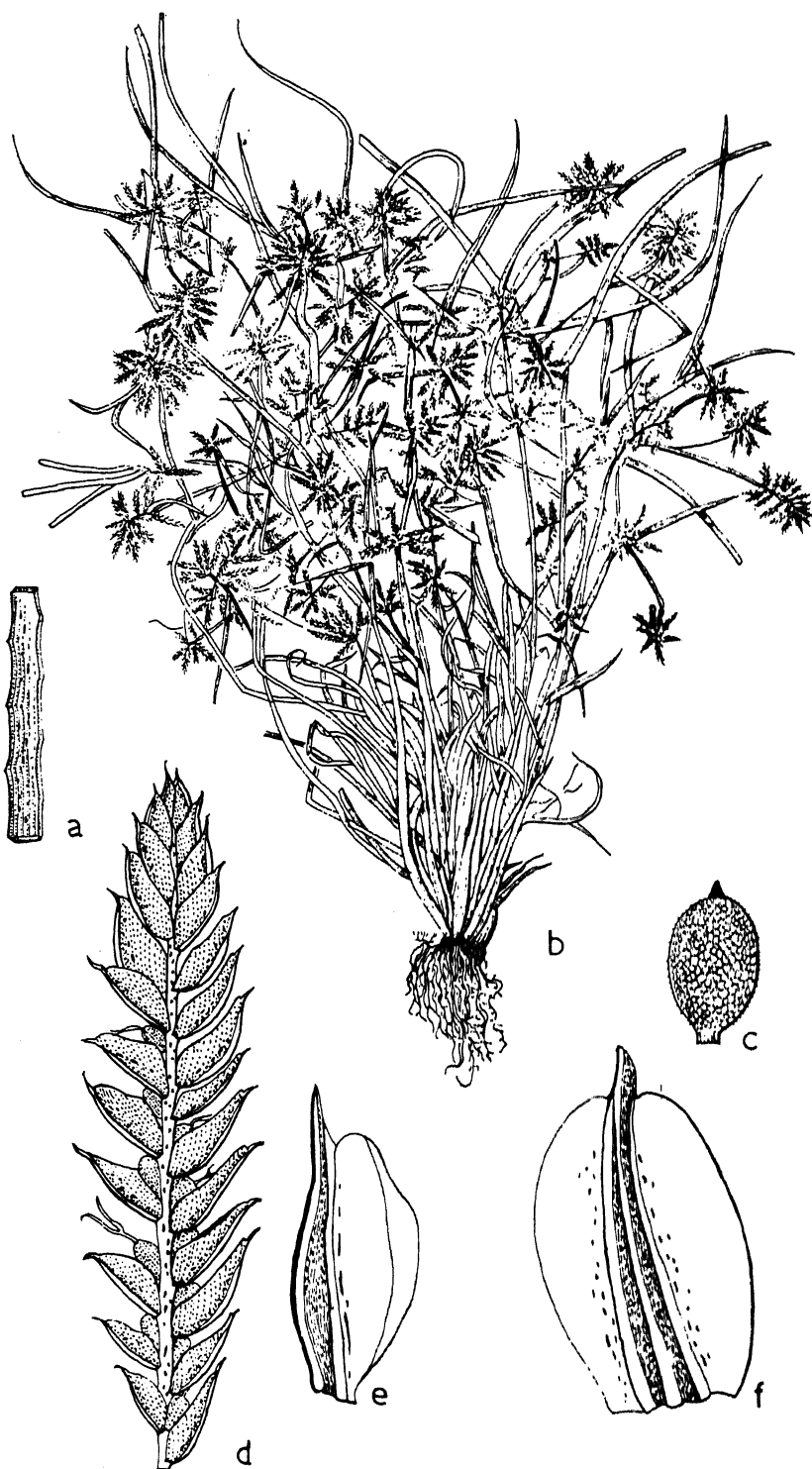
Figure 2. For caption, see page no. 433.
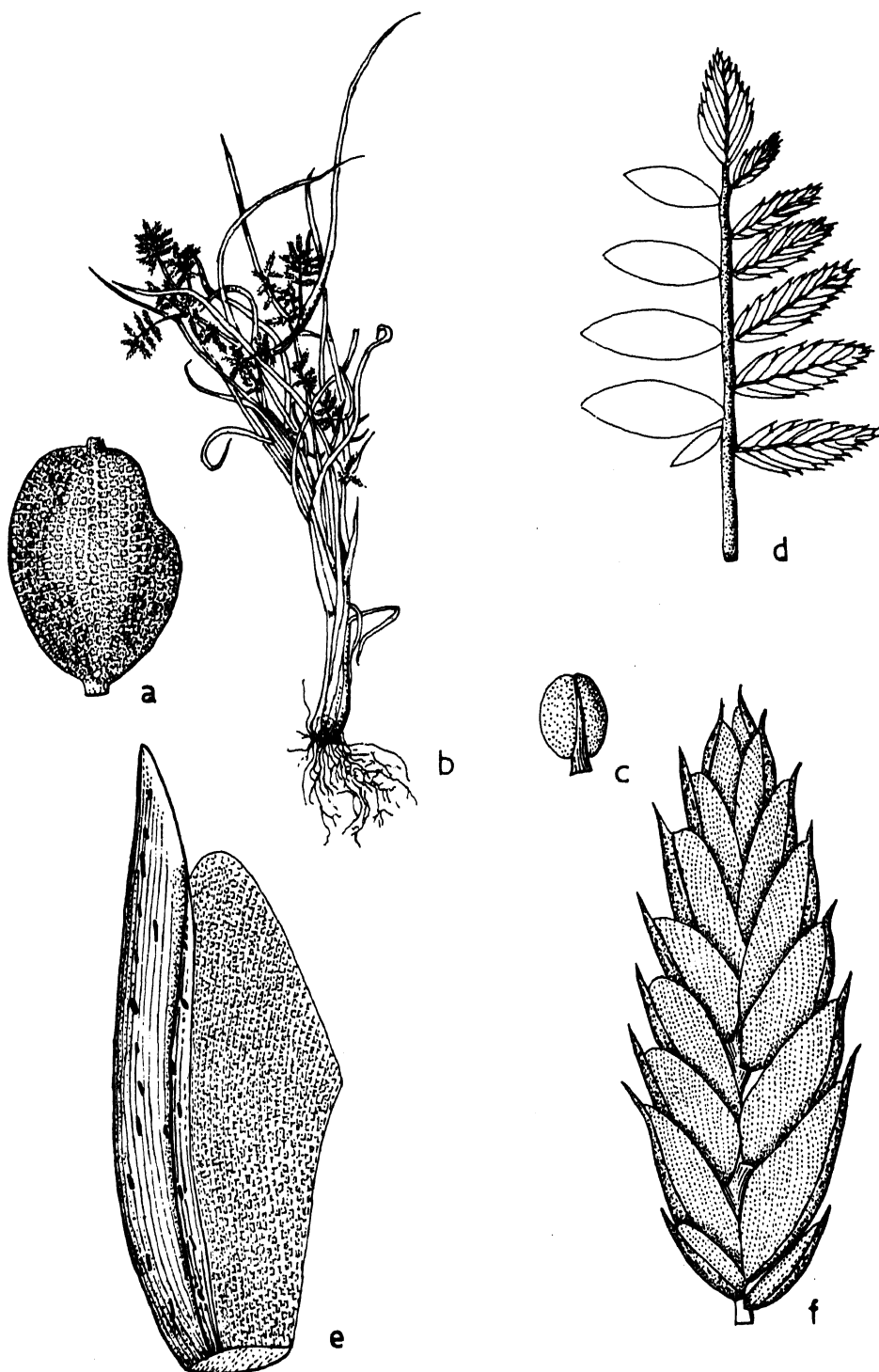
Figure 3. *Pycreus palghattensis* Govind. sp. nov. **a.** Nut (×75). **b.** Habit (×0·5). **c.** Anther (×25). **d.** Spike (×4). **e.** Glume, lateral view (×75). **f.** Spikelet (×15) (Nambiar 12713: type).

glumis compactis paene erectis, nucibus plerumque obovatis paleis bruneis 1/3 pro longitudine glumorum cum apice truncata vel rotundata.

VPK Nambiar 12713, Palghat, Kerala State (type: CAL).

Annual. Roots many, rather thick, brown. Culms somewhat gracile, tufted, 3-ribbed, leafy at base, 6–12 cm tall. Leaves many, flat, distantly scabrid and setaceous towards apex, as long as culms, 1–1·5 mm broad; sheaths brown with many purplish red nerves. Bracts leaf like, obliquely erect, distantly scabrid towards apex, up to 4, the longest 8–9 cm long. Spikes distinct, rectangular, patent at right angles at end of each ray, *c.* 8 mm across. Rays 3–5, each bearing 5–10 (– 15) spikelets, up to 2 cm long. Spikelets elliptic or elliptic ovate, subtumid, remote, usually 10 flowered. 3–3·5 × 1·8–2·2 mm. Rachilla flexuose marked by persistent scales, narrowly winged, excavated. Glumes usually oblong with parallel sides or oblong ovate, almost erect, compact later becoming loose, strongly keeled, mucronate with reddish or pale brown nerveless membranous sides, 1–1·1 (excl. mucro) × 1 mm; keel 3 nerved, green, tannin streaked; mucro erect, 0·4–0·5 mm long. Stamens 2; anther very small, suborbicular or subelliptic, yellow, 0·3–0·4 mm long. Style slender, deeply bifurcated *c.* 0·5 mm long; stigma 2, included, little longer than style. Nut usually elliptic, elliptic ovate or obovate, asymmetric or symmetric, pale brown, hardly apiculate, shortly stipitate, truncate or rounded at apex with granulate surface, 0·4–0·5 mm long and broad, 1/3 length of glumes.

11. Pycreus similinervulosus Govind. sp. nov. (figure 4)

Affinis *Pycreo nervuloso* Kükenth. comb. nov. (= *Cypero nervuloso* Kükenth.) sed ab eo differt culmis gracilibus, foliis pluribus gracilibus gradatim acuminatis angustioribus per culmum, foliis distinctis scabridis ad apicem et culmis aequalibus vel multum longioribus, bracteis foliis similibus culmis aequalibus et distinctis ad apicem scabridis, radiis rigidis erectis oblique erectis longioribus, spicis rectangularibus angustioribus spicis cum multioribus spiculis, glumis oblongis ovatis vel oblongis cum plus minusve enervibus parallelis lateribus, mucrone cum albo bidenticulato apice, nucibus cum manifeste granulata pagina.

Govindarajalu 6012, very common on the way to Orukomban, Poringalkuthu, Kerala State (type: CAL); isotype (MH); paratypes: Govindarajalu 6045, Anakayam, Poringalkuthu, Kerala State (ASSAM); Govindarajalu 6450 A-C, Cheriakanam, Thekkady, Kerala State, not common: 6450 A, B (MH); 6450 C (ASSAM); Govindarajalu 6080, Sholayar, Kerala State, common (BSI); Govindarajalu 6073, Sholayar, Thekkady, Kerala State, common in marshy places (CAL); Govindarajalu 6439, Cheriakanam, Thekkady, Kerala State, common in marshy places (DD); Govindarajalu 6076, Sholayar, Kerala State (DD); Govindarajalu 6089, Sholayar, Kerala State, common on the roadside (BLAT).

Annual. Roots many, fibrous, yellowish or yellowish brown. Culms gracile (rarely setaceous), triquetrous, subcaespitose, 6–25 cm × *c.* 0·5 mm. Leaves usually many, gradually acuminate, distinctly scabrid towards apex, flat, slender (rarely setaceous), usually longer than and overtopping culms, 0·5–0·6 mm broad; sheaths brown or purplish red, distinctly nerved with obliquely truncate mouth. Inflorescence compound, distinctly spicate, rayed with laxly arranged spikelets. Primary rays 3–5, stiff, erect or obliquely erect, (1 –) 5–8 cm long. Spike rectangular consisting of (5 –) 15–20 spikelets (well developed specimens), 2–3 cm across. Involucral bracts 3–4,

Figure 4. *Pycreus similinervulosus* Govind. *sp. nov.* **a.** Anther (×50). **b.** Glume, lateral view (×28). **c.** Rachilla, diagrammatic. **d.** Habit (×0·5). **e.** Glume, spread out (×26). **f and g.** Nuts of different forms (×30). **h.** Epicarpic cells, diagrammatic. **i.** Spikelet (×8) (Govindarajalu 6012; type).

usually as long as culms, leaf like, distinctly scabrid towards apex, slender, obliquely erect, 8–20 cm long. Ochrea tubular, brown with obliquely truncate mouth, *c.* 3 mm long. Spikelets narrowly oblong ovate or narrowly ovate (elliptic) or oblong with parallel sides, acute or subacute, somewhat compressed, 25–40 (− 50) flowered, 8–12 × 2·5–3·0 mm. Rachilla zigzag, visible, winged. Glumes narrowly oblong ovate or oblong with parallel sides, scarious or pale brown, sparsely tannin punctate with 'V' shaped incision at apex, widely patent, obliquely or more or less erect with nerveless sides, strongly keeled, mucronate, 1·5–1·8 (excl. mucro) × (0·5−) 0·8–0·9 mm; keel strong, green, 3 nerved; mucro recurved with colourless bidentate apex, 0·5–0·6 mm long. Stamens 2 with slender persistent filaments; anthers very small, yellow, rounded at apex, elliptic, 0·1–0·2 mm long. Style short, *c.* 0·3 mm long; stigma longer than style. Nuts somewhat variable (ovate, obovate or turbinate) with depressed apex, reddish brown or blackish, minutely apiculate, more or less tumid, shortly stipitate, 1/3 length of glumes, 0·5–0·6 × 0·5 mm; epicarpic surface with vertically arranged rows of prominent granulate markings.

Key to species

1. Spikes globose; rachilla straight, wingless; glumes with nerveless sides; mucro erect; nuts elliptic oblong, oblong ovate or obovate, obtuse, truncate at apex.

   2. Leaves rigid; spikelets linear oblong, usually congested and capitate; rachilla raised, not visible; glumes 2 mm broad, tightly overlapping; stamen 1; nuts 1/3 length of glumes. . . . . *P. pumilus*

   2. Leaves filiform; spikelets oblong ovate, usually sublax, horizontally spreading; rachilla not raised, visible; glumes 1 mm broad, lax; stamens 2; nuts 1/2 length of glumes. . . . . *P. punctatus*

1. Spikes rectangular or ovoid; rachilla zigzag or flexuous, winged; glumes with or without lateral nerves; mucro erect or recurved; nuts variable in shape.

   3. Spikes *c.* 8 mm across with 5–10 spikelets; spikelets elliptic or elliptic ovate, *c.* 10 flowered, 3–3·5 mm × 1·8–2·2 mm; rachilla flexuous, scaly; glumes compact without lateral nerves, retuse, 1–1·1 × 1 mm; mucro erect, entire, 0·4–0·5 mm long; nuts truncate or rounded at apex. . . . . : . . : . . . . . *P. palghattensis*

   3. Spikes 20–30 mm across with 15–20 spikelets; spikelets narrowly oblong ovate, elliptic ovate or oblong with parallel sides, 25–40 (− 50) flowered, 8–12 × 2·5–3 mm; rachilla zigzag, smooth; glumes obliquely spreading or almost erect with 1–2 lateral nerves and 'V' shaped incision at apex, 1·5–1·8 × 0·5–0·6 mm; mucro recurved, bidenticulate, 0·5–0·6 mm long; nuts with depressed apex. . . . . *P. similinervulosus*

   4. Spikes 6–15 mm across with 5 10 spikelets; spikelets oblong gradually widening towards apex, 16–18 flowered, 3·5–7 mm long rachilla flexuous; glumes steeply oblique with nerveless sides 1 mm broad; mucro recurved, 0·4 mm long . . . . *P. membranaceus*

Acknowledgements

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Figure 1. **a, b, i–l.** *Pycreus pumilus* (L.) Domin; **a and b.** Nuts of different forms (× 21). i. Spikelet (× 8). **j.** Glume, lateral view (× 32). **k.** Glume apical portion, diagrammatic. **l.** Glume spread out (× 27) (Govindarajalu 5480). **c–h.** *Pycreus membranaceus* (Vahl) Govind. comb. nov. **c.** Spikelet (× 10). **d.** Glume, spread out (× 22). **e.** Glume, lateral view (× 22). **f.** Anther (× 13). **g.** Nut (× 24). **h.** Habit (× 0·5) (Govindarajalu 6076).

Figure 2. *Pycreus punctatus* (Roxb.) Govind. *comb. nov.* **a.** Rachilla, diagrammatic. **b.** Habit (× 0·5). **c.** Nut (× 25). **d.** Spikelet (× 8). **e.** Glume, lateral view (× 33). **f.** Glume, spread out (× 38) (Venugopal 13934).

Effect of cement kiln dust pollution on black gram (*Vigna mungo* (L.) Hepper)

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MS submitted 2 July 1990

Abstract. Effect of cement kiln dust pollution on black gram (*Vigna mungo*) has been studied by comparing plants of polluted as well as from non-polluted areas. Due to cement kiln dust accumulation on exposed parts of the plant, there was a decrease in height, phytomass, net primary productivity and chlorophyll content. Quantitative estimations and histo-chemical localization indicate lowering of metabolites in dusted plants as compared to control one. In polluted plants, damaged leaves show increase in stomatal index and trichome frequency and decrease in stomatal frequency. Cement kiln dust accumulation on plant surface showed decrease in the number and size of flowers which finally affected the yield to a great extent in the dusted plants.

Keywords. Black gram; cement kiln dust; phytomass; pollution; yield.

1. Introduction

Cement kiln dust is considered as one of the most dangerous dusts of industrial origin since it not only forms a crust, but also enters into a chemical reaction with the atmospheric moisture and is thus, chemically active (Czaja 1962). The cement kiln dust is reported to be harmful to vegetation, causing considerable reduction in agricultural production, primarily affecting fertilization and starch production (Darely 1966). Studies on the phytotoxic effects of different kinds of particulate pollutants has been carried out by a number of workers from time to time. The particulate dust falling on the leaves is said to cause foliar injuries, reduction in yield, changes in the rate of photosynthesis, transpiration and uptake and accumulation of mineral elements from soil (Lerman and Darely 1975). *Vigna mungo* (L.) Hepper commonly known as black gram, an economically important Fabaceae member has been chosen to study the various effects of cement kiln dust pollution in detail, since there is a lacuna in the studies of particulate pollution on economic crop plants.

2. Materials and methods

Seeds of *V. mungo* were procured from the market. Seedlings were raised in the University botanical garden in a plot of 16 sq. m size divided into two halves of equal size. The seeds were sown at 15 cm intervals in rows with a distance of 10-cm between two rows. Plants grown in one plot served as control, whereas those in the other plot were uniformly dusted with $10 \, \text{g m}^{-2} \, \text{day}^{-1}$ cement kiln dust. Five plants were sampled at each sampling time at 20 days interval from both control and experimental plots and washed thoroughly in water, rinsed in distilled water and blotted with filter paper prior to further use. The height of plant were recorded during the experiment period. Net primary productivity (NPP) was determined by

dividing the total phytomass value by the age of the plant. Leaves were sampled to determine parameters such as chlorophyll content (Mac Lachlan and Zalik 1963), proteins (Layne 1957), starch (McCreddy *et al* 1950), total sugars (Dubois *et al* 1956), reducing sugars (Miller 1972), lipids (Bragdon 1951; Folch *et al* 1957), amino acids (Moore and Stein 1948), total phenols (Bray and Thorpe 1954) and orthodihydroxy phenols (Arnow 1937). Epidermal peels were obtained by direct peeling method. Peels were then used for epidermal studies and also for histochemical tests such as protein (Chapman 1975), starch (Johansen 1940), lipid (Bronner 1975), peroxidase (Graham and Karnovsky 1966), succinic dehydrogenase (Pearse 1972) and cytochromeoxidase (Burstone 1959).

3. Results

3.1 *Morphological parameters*

The results obtained of cement kiln dust dusted and non-polluted plants were compared as regards height, number of leaves per plant, size of leaves, thickness of lamina, branching of stem, phytomass, NPP, number of flowers and yield (table 1).

3.1a *Height of plant*: The height of control and dusted plants after 20 and 120 days was 16 and 157 cm, and 13 and 100 cm respectively. There is a reduction of 36% in height of dusted plants after 120 days.

3.1b *Root length*: The root length of control and dusted plants after 20 and 120 days was 10·2 and 30·0 cm, and 8·6 and 20·0 cm respectively. There is a reduction of 33·33% in root length of dusted plants after 120 days. The decrease in root length of dusted plants also affects the root nodules. There is a decrease of 50% in root nodules of dusted plants.

3.1c *Branches*: Branching was noticed only after the plants attained the age of 40 days. The branches of control and dusted plants after 40 and 120 days were 3 and 25, and 0 and 10 respectively. There is a significant reduction in number of branches of dusted plants after 120 days. This indicates that in control plants the branching started earlier than dusted plants.

3.1d *Leaves*: Number of leaves in the control and dusted plants after 20 and 120 days were 4 and 120, and 3 and 50 respectively. A reduction of 55·33% in number of leaves in dusted plants after 120 days was recorded. Reduced number of leaves in dusted plants was also associated with the conspicuous decrease in the thickness of lamina and leaflet size.
    Cement kiln dust was found accumulated on both surfaces of the leaflets. However, accumulation of dust particles were more on adaxial surface compared to abaxial surface. Leaflets retained 2·05 mg of dust per sq. cm on their exposed surfaces. Leaflets get reduced in size and become curved. The texture of the leaflets become brittle.

3.1e *Phytomass*: Phytomass values showed a decrease in dusted than control plants. There is a reduction of 62·3% in stem, 64·0% in leaf and 63·67% in root

Table 1. Morphological parameters of control and dusted plants of *V. mungo*.

Age of plant		Height of plant	Root length	Branches	Leaves	Phytomass	NPP	Root/shoot ratio	Flowers	Fruits
20	C	16	10·2	—	4	3·873	0·194	0·64	—	—
	D	13	8·6	—	3	3·368	0·168	0·66	—	—
40	C	36·5	18·5	3	27	31·138	0·778	0·51	—	—
	D	26	14	0	10	20·749	0·521	0·54	—	—
60	C	50	23·5	17	70	90·810	1·514	0·47	—	—
	D	33·9	16·6	6	34	51·862	0·864	0·49	—	—
80	C	120	26·5	20	100	197·090	2·464	0·22	5	—
	D	79	18	8	50	105·729	1·322	0·23	3	—
100	C	150	29	25	130	198·796	1·988	0·19	30	10
	D	97	19·6	10	70	86·642	0·866	0·20	15	5
120	C	157	30	25	120	141·206	1·177	0·19	90	80
	D	100	20	10	50	51·922	0·433	0·20	45	40

Average of 5 replicates. C, Control; D, dusted.

phytomass in dusted plants after 120 days. Total phytomass values exhibited an increase up to 80 days of growth. After 120 days a reduction of 63·23% in total phytomass of dusted plants is noticed.

3.1f   *NPP*: The NPP values of control and dusted plants after 20 and 120 days were 0·194 and 1·177 g, and 0·168 and 0·433 g respectively. There is a reduction of 63·21% in NPP of dusted plants after 120 days. The significant decrease in NPP of dusted plants is also correlated with decrease in phytomass.

3.1g   *Root/shoot ratio*: It showed a continuous decrease with an increase in age of plant in control and dusted plants. The ratio of control and dusted plants after 20 and 120 days was 0·64 and 0·19, and 0·66 and 0·20 respectively. Root/shoot ratio of dusted plants after 120 days was enhanced by 5%.

3.1h   *Flowers*: Flowering started after 60 days of growth period. The number of flowers in control and dusted plants after 80 and 120 days was 5 and 90, and 3 and 45 respectively. There is a 50% reduction in number of flowers of dusted plants at 120 days age.

3.1i   *Yield*: Dusted plants at 120 days age showed 50% reduction in number of flowers. This leads to a 50% reduction in fruit set and ultimately yield. Fruiting started after 80 days of growth period. The number of mature fruits in control and dusted plants after 100 and 120 days was 10 and 80, and 5 and 40 respectively.

3.2   *Biochemical estimations* (table 2).

3.2a   *Chlorophyll content*: The total chlorophyll content, chlorophyll *a* and chlorophyll *b* exhibited an increase with an increase in age of plant up to 80 days growth which later decreased. The percentage of reduction in dusted plants chlorophylla *a* and total chlorophyll content increased with an increase in age of plant, however, chlorophyll *b* decreased with an increase in age of plant.

The total chlorophyll content of control and dusted plants after 20 and 80 days was 1·555 and 2·245 mg/g, and 1·391 and 1·785 mg/g respectively which later decreased to 0·762 and 0·583 mg/g in control and dusted plants after 120 days. There was 24·49% reduction in total chlorophyll content of dusted plants after 120 days. The decrease in total chlorophyll content of dusted plants is also correlated with the decrease in chlorophyll *a* and chlorophyll *b* contents.

3.2b   *Total protein content*: Total protein content of control and dusted plants after 20 and 80 days was 0·519 and 0·258 mg/g, and 0·135 and 0·191 mg/g respectively, which later decreased to 0·202 and 0·135 mg/g in control and dusted plants after 120 days. Total protein content of dusted plants after 120 days is decreased by 33·16%.

3.2c   *Starch content*: The starch content of control and dusted plants after 20 and 80 days was 1·305 and 4·007 mg/g, and 1·188 and 3·250 mg/g respectively, which later decreased to 2·691 and 1·832 mg/g in control and dusted plants after 120 days. Reduction of 31·92% starch content was observed in dusted plants after 120 days.

Table 2. Biochemical parameters of control and dusted plants of *V. mungo*.

Age of plant (days)		Total chlorophyll content (mg/g)	Total protein content (mg/g)	Starch content (mg/g)	Total sugar content (mg/g)	Reducing sugar content (mg/g)	Lipid content (mg/g)	Amino acid content (mg/g)	Total phenol content (mg/g)	O.D. phenol content (mg/g)
20	C	1·555	0·159	1·305	5·145	0·827	9·390	1·031	0·702	0·174
	D	1·391	0·135	1·188	4·676	0·775	8·710	0·893	0·641	0·161
	PR	10·55	15·09	8·97	9·12	6·28	7·24	13·38	8·69	7·47
40	C	1·722	1·75	2·509	16·196	3·394	13·170	1·296	0·749	0·291
	D	1·495	0·146	2·182	14·374	2·893	10·470	1·090	0·688	0·265
	PR	13·18	16·57	13·03	11·25	14·76	20·50	15·89	10·81	8·93
60	C	1·925	0·198	2·818	33·196	5·173	17·190	2·302	0·840	0·365
	D	1·605	0·151	2·291	26·790	4·136	16·370	1·844	0·719	0·328
	PR	16·62	23·73	18·70	19·29	20·05	4·77	19·89	14·40	10·13
80	C	2·245	0·258	4·007	45·976	7·115	19·470	3·039	1·407	0·479
	D	1·785	0·191	3·250	34·523	5·209	21·341	2·350	1·179	0·415
	PR	20·49	25·96	18·89	24·91	26·79	8·77	22·67	16·20	13·36
100	C	1·930	0·215	3·981	42·556	6·280	16·870	2·786	0·851	0·379
	D	1·529	0·156	2·852	30·172	4·271	19·160	2·082	0·694	0·313
	PR	20·78	27·44	28·35	29·10	32·00	11·95	25·27	18·45	17·41
120	C	0·762	0·202	2·691	36·125	4·151	13·180	1·600	0·769	0·328
	D	0·583	0·135	1·832	23·512	2·637	17·396	1·145	0·575	0·263
	PR	23·49	33·16	31·92	34·91	36·47	24·23	28·44	25·23	19·82

Average of 5 replicates. C, Control; D, dusted; PR, per cent reduction.

3.2d *Sugar content*: Total sugar and reducing sugar content exhibit an increase with an increase in age of control and dusted plants. Reduction of 34·91% total sugar content and 36·47% reducing sugar content was observed in dusted plants after 120 days.

3.2e *Lipid content*: Quantitative values of lipid content in control plants is higher than that of dusted plants up to 60 days. Lipid content becomes higher in dusted than control plants after 60 days. Lipid content of dusted plants after 120 days is increased by 24·23%.

3.2f *Amino acid content*: The amino acid content of control and dusted plants after 20 and 80 days was 1·031 and 3·039 mg/g, and 0·893 and 2·350 mg/g respectively, which later decreased to 1·600 and 1·145 mg/g in control and dusted plants after 120 days. Amino acid content of dusted plants after 120 days is reduced by 28·44%.

3.2g *Phenolic content*: The quantitative values of total phenols and OD phenols increased up to 80 days of growth period with an increase in age of plant which later decreased. The total phenol and OD phenol content of dusted plants after 120 days were reduced by 25·23 and 19·82% respectively.

3.3 Histochemistry

Histochemical localization of proteins and starch showed a decreased activity, whereas lipids showed increased activity in dusted plants as compared to control plants. Localization of enzymes like succinic dehydrogenase, cytochrome oxidase and peroxidase revealed less activity in dusted plants with reduction in metabolites.

3.4 Epidermal studies

Leaves of *V. mungo* are amphistomatic. Epidermal cells are polygonal with wavy anticlinal walls. Epidermal cell frequency decreases in polluted plants in comparison with control plants. Epidermal cell frequency is more on abaxial surface than adaxial on both control and dusted plants (table 3).

Table 3. Epidermal features of non-polluted and polluted plants of *V. mungo*.

		Epidermal cell frequency	Stomatal index	Stomatal frequency	Paracytic	Anomocytic	Abnormal	Trichome frequency	Eglandular trichomes	Glandular trichome
Ad	NP	496	17·6	106	96·4	3·6	—	41	74·8	25·2
	P	315	22·4	91	93·4	2·4	4·2	65	72·8	27·2
Ab	NP	797	24·6	260	97·0	3·0	—	24	28·6	71·4
	P	642	27·4	242	94·3	4·2	1·5	30	36·6	63·4

Average value of 5 replicates.
Ad, Adaxial; Ab, abaxial; NP, non-polluted; P, polluted.

In polluted plants few abnormal stomata were observed. Stomatal frequency was reduced in polluted plants, while stomatal index increased in polluted plants. The stomatal index was more on abaxial surface in both control and dusted plants (table 3).

Trichomes were observed on both the surfaces, however, their density was more on adaxial than on abaxial surface. Trichome frequency increases in polluted plants. Eglandular and glandular clavate type of trichomes are observed in both surfaces. Eglandular trichomes are dominent on adaxial surface, while glandular clavate type is dominent on abaxial surface of both control and dusted plants (table 3).

4. Discussion

Among particulate air pollutants, cement kiln dust is a potential phytotoxic pollutant in the vicinity of a cement factory. In the present study, formation of a hard thick encrustation of cement kiln dust on the plant surfaces was observed in cement kiln dusted plants (Oblisami *et al* 1978; Armbrust 1986).

In the present study, cement kiln dust was found to reduce height of plant in dusted plants. Reduction in height of plant in responce to environmental pollution due to decreased photosynthesis per unit leaf area and/or enhanced leaf senescence, increased respiration. The inhibition in growth is due to reduced intensity of light energy available for photosynthesis through coatings of leaves (Mishra 1982; Emanuelson 1984; Indhirabai *et al* 1988, 1989). Root length as well as nodulation in roots is also reduced due to increased pH of soil and the presence of calcium in dust which is added to soil. The number of branches in dusted plants was found to be decreased. The height of plant and internodal elongation were reduced by cement kiln dust pollution (Indhirabai *et al* 1988, 1989). Due to formation of cement crust on leaves, polluted plants showed a decrease in lamina thickness and size of leaf.

The phytomass and NPP values of cement kiln dust dusted plants showed lesser values than control plants. This indicates a reduction in photosynthesis of dusted plants (Singh and Rao 1981). Singh and Rao (1981) reported that changes in the root/shoot ratio of dusted plants showed a trend similar to that of control plants. The values in dusted plants were always higher than control plants (Borka 1980).

Under the effect of cement kiln dust the acidic secretion of stigma turned into alkaline, a condition which is unfavourable for pollen germination which leads to poor fertilization and yield (Borka 1986). From gross morphological measurements of control and dusted plants it is quite apparent that dusted environmental condition had an adverse effect on the vegetative growth, flowering and fruiting potential of plants thus, indicating a considerable reduction in the productivity of the plant.

Cement kiln dust, on entering into leaf tissues, the chemically active solution caused partial denaturation of the chloroplasts and a decrease in pigment content in the cells of damaged leaves (Borka 1986). Higher levels of cement kiln dust pollution considerably decrease the growth and metabolic activities. One of the most characteristic biochemical feature of cement kiln dust dusted plants is a reduction in total chlorophyll content (Singh 1979; Singh and Rao 1981; Pawar *et al* 1982; Rajachidambaram 1983).

Present observation on the reduction in protein content in dusted plants is parallel to that of many workers (Prasad 1980; Agrawal 1982; Pawar *et al* 1982). It

thus appears that the total protein content is also a suitable indicator of particulate pollution level. Closing of stomata not only prevented the inward diffusion of necessary amount of CO_2, but because of higher temperature caused by reduced transpiration, inhibits the phosphorylation of sugars and thereby the removal of starch from the site of origin. The increase in lipid content in dusted plants appears to be an adaptation by plants against pollution (Malhotra and Khan 1978).

Chemical information can be best expressed in morphological terms through histochemical methods (Malik and Singh 1980). Constantinidou and Kozlowski (1979) reported that *Ulmus* seedlings exposed to air pollutants showed a decrease in carbohydrates, proteins and lipid contents which also is evident in dusted plants. The decrease of these metabolites leads to reduction in growth and finally yield. Percy and Riding (1981) reported similar results in *Pinus* needles.

A decrease in the number of stomata in leaf epidermis in polluted plants indicates a favourable adaptation (Sharma and Butler 1973; Yunus and Ahmed 1980) to regulate the transpiration as well as the limited and controlled entry of harmful pollutants into plant tissues. The more number of trichomes help in protecting the leaf from direct exposure to sun rays, thus lowering the leaf temperature and reducing the rate of metabolic reaction associated with the destruction of plant tissues.

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SUBJECT INDEX

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Announcement of Merger

The Council of the Indian Academy of Sciences has decided to merge three of the biology journals published by the Academy: *Proceedings*: *Plant Sciences*, *Proceedings*: *Animal Sciences* and the *Journal of Biosciences*. Consequently, this will be the **last issue** of the *Proceedings*: *Plant Sciences*. For various practical reasons, it has been decided to retain the name *Journal of Biosciences* for the merged journal. This will be a quarterly and the first two issues combined will appear in June 1991. Since this is an important decision reversing an earlier one taken in 1978 it is appropriate to briefly mention some of the reasons underlying this merger.

Since the Academy was founded in 1934, its main activity has been the publication of scientific journals. The *Proceedings of the Indian Academy of Sciences* appeared in two sections: Section A was devoted to Physical Sciences and Section B to Life Sciences. In 1978, following the worldwide trend, the *Proceedings* were split into several subjectwise journals. In particular, Section B was split into *Proceedings*: *Plant Sciences*, *Proceedings*: *Animal Sciences and Proceedings*: *Experimental Biology*. The section on Experimental Biology was renamed the *Journal of Biosciences* in 1979. At first sight, it seems like a retrograde step to merge these three journals once again, particularly in the light of the present fashion and preference to have more and more specialized journals. But the main motivation for the rethinking is the following. It is well-known that the overwhelming majority of the better papers published by Indian scientists find their way into journals published abroad. Given this trend it has been particularly difficult to maintain high standards with **four** specialized journals in Biology (with 19 issues per year between them). Thus, sadly, the earlier hope that specialized journals are more likely to attract good papers than the *Proceedings* (which covered all of biology) has not been fulfilled. In the ultimate analysis, however, our priority is to publish papers of high quality. Indeed, in several editorials published in *Current Science* before the Academy was founded, C V Raman argued that the main objective of the Academy would be to publish journals where the more important results of the Indian scientists would appear, rather than be exported. Only then, he argued, can the Indian scientific community gain an international recognition and be freed from a position of semi-dependence. Seized with the problem of the urgent need to revitalize our journals, the Council of the Academy initiated several discussion meetings. The eventual decision to merge these three journals emerged from these meetings between several active biologists and the concerned Editors. Since the *Journal of Genetics* had a very different historical origin, it was felt that it should continue as an independent journal with a somewhat different perspective and character.

The new *Journal of Biosciences* will, in a sense, have a broader scope than the three separate journals that are being merged and will include all areas of biology such as Molecular Biology, Genetics, Developmental Biology, Biophysics, Biochemistry, Immunology, Endocrinology, Medical Biology, Neurobiology, Ecology, Physiology, Ethology, Evolutionary Biology, Environmental Biology, Sensory Biology etc. Consequently a new Editorial Board is being constituted

whose composition will reflect this enlarged scope. This reconstitution will also give an opportunity to a new group of people to share in this important responsibility.

On behalf of the Council and the Fellowship of the Academy I wish to profusely thank all the members of the outgoing Editorial Board for their commitment and dedication to this journal during difficult times. Very special thanks are due to Prof. C V Subramanian, one of the doyens of Plant Sciences in India, for shouldering the responsibility of Editing this journal for more than a decade and for his untiring efforts to sustain this journal.

G Srinivasan
Editor of Publications